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- (71) Applicants (for all designated States except US): **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). **DGI BIOTECHNOLOGIES** [US/US]; 40 Talmadge Road, Edison, NJ 08818-0424 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **PILLUTLA, Renuka** [IN/US]; 10 Pope Road, Bridgewater, NJ 08807 (US). **BRISSETTE, Renee** [US/US]; 2676 Wildberry Court, Edison, NJ 08817 (US). **BLUME, Arthur, J.** [US/US]; 11 Walden Drive, Annandale, NJ 08801 (US). **SCHÄFFER, Lauge** [DK/DK]; Hormemansgade 12, 1, DK-2100 Copenhagen Ø (DK). **BRANDT, Jakob** [DK/DK]; Tjoernevangen 27, DK-2700 Broenshoej (DK). **GOLDSTEIN, Neil, I.** [US/US]; 26 Kendal Avenue, Maplewood, NJ 07040 (US). **SPETZLER, Jane**
- (74) Agent: **SONNENFELD, Kenneth, H.**; Morgan & Finnegan, LLP, 345 Park Avenue, New York, NY 10154 (US).
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(54) Title: INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites, which are present on insulin and insulin-like growth factor receptors, and which selectively bind the peptides of this invention. As agonists, certain of the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonists may also be developed as therapeutics.

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INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

This application is a continuation-in-part of U.S. Application Serial No. 09/538,038 filed September 24, 2002, which is a continuation-in-part of U.S. 5 Application Serial No. 09/538,038 filed March 29, 2000, which is a continuation-in-part of U.S. Application Serial No. 09/146,127, filed September 2, 1998, both of which are incorporated by reference in their entirety.

I. FIELD OF THE INVENTION

10 This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling
15 whereby useful molecular models are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of 20 glucose levels in the body. This effect occurs predominantly in liver, fat, and muscle tissue. In the liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat tissue, insulin stimulates glucose uptake, storage, and metabolism. Defects in glucose utilization are very common in the population, giving rise to 25 diabetes.

Insulin initiates signal transduction in target cells by binding to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of IR, which are transmitted across the cell membrane and result in activation of the

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receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of tyrosine kinase of IR, and the binding of soluble effector molecules that contain SH2 domains such as phosphoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase C γ to IR (Lee and Pilch, 1994, *Am. J. Physiol.* **266**:C319-C334).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and repair. Recently, IGF-1 has been implicated in various forms cancer including prostate, breast, colorectal, and ovarian cancers. It is similar in size, sequence, and structure to insulin, but has 100-1,000-fold lower affinity for IR (Mynarcik et al., 1997, *J. Biol. Chem.* **272**:18650-18655).

Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll et al., 1997, *Diabetes* **46**:1453-1458; Crowne et al., 1998, *Metabolism* **47**:31-38), amyotrophic lateral sclerosis (Lai et al., 1997, *Neurology* **49**:1621-1630), and diabetic motor neuropathy (Apfel and Kessler, 1996, *CIBA Found. Symp.* **196**:98-108). Other potential therapeutic applications of IGF-1, such as osteoporosis (Canalis, 1997, *Bone* **21**:215-216), immune modulation (Clark, 1997, *Endocr. Rev.* **18**:157-179) and nephrotic syndrome (Feld and Hirshberg, 1996, *Pediatr. Nephrol.* **10**:355-358), are also under investigation.

A number of studies have analyzed the role of endogenous IGF-1 in various disease states. Interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995, *Endocrinol.* **136**:5485-5492; Figueroa et al., 1995, *J. Clin. Endocrinol. Metab.* **80**:3476-3482; Torring et al., 1997, *J. Urol.* **158**:222-227). Additionally, elevated serum IGF-1 levels correlate with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan et al., 1998, *Science* **279**:563-566). Recent studies have indicated a connection between IGF-1 levels and other cancers such as breast, colorectal, and

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ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1 receptor (IGF-1R; reviewed in Conover, 1996, *Endocr. J.* **43S**:S43-S48 and Rajaram *et al.*, 1997, *Endocr. Rev.* **18**:801-831). Interestingly, PSA 5 has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (Cohen *et al.*, 1992, *J. Clin. Endocrinol. Metab.* **75**:1046-1053; Cohen *et al.*, 1994, *J. Endocrinol.* **142**:407-415; Lilja, 1995, *J. Clin. Lab. Invest. Suppl.* **220**:47-56). Clearly, regulation of IGF-1R activity can play an important role in several disease states, indicating that 10 there are potential clinical applications for both IGF-1 agonists and antagonists.

IGF-1R and IR are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are composed of two α and two β subunits which form a disulfide-linked 15 heterotetramer (β - α - α - β). These receptors have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire α subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the 20 tyrosine kinase domain. Another family member is insulin-related receptor (IRR), for which no natural ligand is known.

While similar in structure, IGF-1R and IR serve different physiological functions. IR is primarily involved in metabolic functions whereas IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can 25 induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to IGF-1R, and IGF-1 its metabolic effects through IR, remains controversial (De Meyts, 1994, *Horm. Res.* **42**:152-169).

30 IR is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single

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polypeptide chain and proteolytically cleaved to yield a disulfide-linked monomer α - β insulin receptor. Two α - β monomers are linked by disulfide bonds between the α -subunits to form a dimeric form of the receptor (β - α - α - β -type configuration). The α subunit is comprised of 723 amino acids, and it
5 can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine rich region (amino acids 156-312) (Ward *et al.*, 1995, *Prot. Struct. Funct. Genet.* **22**:141-153). Many determinants of insulin binding seem to reside in the α -subunit. A unique feature of IR is that it is dimeric in the absence of ligand.
10 The sequence of IR is highly homologous to the sequence of IGF-1R. The sequence identity level varies from about 40% to 70%, depending on the position within the α -subunit. The three-dimensional structures of both receptors may therefore be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998, *Nature* **394**:395-399). The L domains consist of a single-stranded right-handed β -helix (a helical arrangement of β -strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.
15

The β -subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The
20 extracellular domain is linked by disulfide bridges to the α -subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994, *Nature* **372**:746-754).

To aid in drug discovery efforts, a soluble form of a membrane-bound
25 receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability
30 to bind insulin was similar to that of the full-length holoreceptor.

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- IGF-1 and insulin competitively cross-react with IGF-1R and IR. (L. Schäffer, 1994, *Eur. J. Biochem.* **221**:1127-1132). Despite 45% overall amino acid identity, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 5 3 orders of magnitude lower than that for the cognate receptor (Mynarcik, et al., 1997, *J. Biol. Chem.* **272**:18650-18655). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands (Blakesley et al., 1996, *Cytokine Growth Factor Rev.* **7**(2):153-9).
- 10 Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, where the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin (Blakesley, 1996). The C region of human IGF-1 appears to be required for high affinity binding to IGF-1R (Pietrzkowski et al., 1992, *Cancer Res.* **52**(23):6447-51).
- 15 20 Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R (Pietrzkowski et al., 1992). A further distinction between the two hormones is that, unlike insulin, IGF-1 has very 25 weak self-association and does not hexamerize (De Meyts, 1994).

The α -subunits, which contain the ligand binding region of IR and IGF-1R, demonstrate between 47-67% overall amino acid identity. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid identity.

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Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α -subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ (T. Kjeldsen et al., 1991, *Proc. Natl. Acad. Sci. USA* **88**:4404-4408; A.S. Andersen et al., 1992, *J. Biol. Chem.* **267**:13681-13686). For example, the cysteine-rich domain of the IGF-1R was determined to be essential for high-affinity IGF binding, but not insulin binding. When amino acids 191-290 of IGF-1R region was introduced into the corresponding region of the IR (amino acids 198-300), the modified IR bound both IGF-1 and insulin with high affinity. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the modified IGF-1R bound to IR but not IGF-1.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding for either IR or IGF-1R. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region, residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin (Williams et al., 1995). When these residues are introduced into the equivalent site of IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding (Mynarcik et al., 1996, *J. Biol. Chem.* **271**(5):2439-42; C. Kristensen et al., 1999, *J. Biol. Chem.* **274**(52):37351-37356).

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Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to IGF-1R but that those contacts differ from those that insulin utilizes to bind to IR (Mynarcik et al., 1997). Hence, the data in the literature has led one 5 commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems" (De Meyts, 1994).

Based on data for binding of insulin and insulin analogs to various 10 insulin receptor constructs, a binding model has been proposed. This model shows insulin receptor with two insulin binding sites that are positioned on two different surfaces of the receptor molecule, such that each alpha-subunit is involved in insulin binding. In this way, activation of the insulin receptor is believed to involve cross-connection of the alpha-subunits by insulin. A 15 similar mechanism may operate for IGF-1R, but one of the receptor binding interactions appears to be different (Schäffer, 1994, *Eur. J. Biochem.* 221:1127-1132).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious 20 and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth. It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer 25 selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures that mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as 30 therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited

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stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, to date, none of these efforts has resulted in finding an effective drug replacement.

5 Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996, *Proc. Natl. Acad. Sci. USA* 93:7381-7386) reported the isolation of a monomer antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage-
10 panning procedures.

Wrighton *et al.* (1996, *Science* 273:458-464) and Livnah *et al.* (1996, *Science* 273:464-471) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor
15 antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimer peptides that enabled the dimerization two EPO receptors.

WO 96/04557 reported the identification of peptides and antibodies
20 that bound to active sites of biological targets, which were subsequently used in competition assays to identify small molecules that acted as agonist or antagonists at the biological targets. Renchler *et al.* (1994, *Proc. Natl. Acad. Sci. USA* 91:3623-3627) reported synthetic peptide ligands of the antigen binding receptor that induced programmed cell death in human B-cell lymphoma.
25

Most recently, Cwirla *et al.* (1997, *Science* 276:1696-1698) reported the identification of two families of peptides that bound to the human thrombopoietin (TPO) receptor and were competed by the binding of the natural TPO ligand. The peptide with the highest affinity, when dimerized by
30 chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand.

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III. SUMMARY OF THE INVENTION

This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR and/or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules that bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

In one aspect of this invention, large numbers of peptides have been screened for their IR and IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. Several generic amino acid sequences are disclosed which bind IR and/or IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

- a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid (Formula 1; Group 1; A6 motif);
- b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids (Formula 2; Group 3; B6 motif);

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c. $X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21}$ wherein X_{14} , and X_{17} are hydrophobic amino acids, X_{15} , X_{16} , X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids (Formula 3; reverse B6; revB6).

d. $X_{22} X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} X_{34} X_{35} X_{36} X_{37} X_{38} X_{39} X_{40} X_{41}$ wherein X_{22} , X_{25} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} , X_{40} , and X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X_{25} and X_{40} X_{31} and X_{32} are small amino acids (Formula 4; Group 7; E8 motif).

e. $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} may be any amino acid, X_{43} , X_{46} , X_{49} , X_{50} , X_{54} are hydrophobic amino acids, X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51} , X_{52} and X_{57} are small amino acids (Formula 5; mini F8 motif).

f. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} , and X_{81} may be any amino acid; X_{63} , X_{70} , X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop (Formula 6; Group 2; D8 motif).

g. $H X_{82} X_{83} X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92}$ wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine, serine or threonine, X_{85} is a polar amino acid, X_{86} , X_{88} , X_{89} and X_{90} are any amino acid, and X_{87} , X_{91} and X_{92} are an aliphatic amino acid (Formula 7).

h. $X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} , and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104} , X_{105} and X_{106} and at least one of X_{112} , X_{113} and X_{114} are cysteine (Formula 8); and

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i. an amino acid sequence comprising the sequence JBA5: DYK DLCQ SWG VRIG WL AGL CP KK (SEQ ID NO:1541) or JBA5 minus FLAG® tag and terminal lysines: LCQ SWG VRIG WL AGL CP (SEQ ID NO:1542) (Formula 9).

5 j. W X₁₂₃ G Y X₁₂₄ W X₁₂₅ X₁₂₆ (SEQ ID NO:1543) wherein X₁₂₃ is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X₁₂₄ is any amino acid, but preferably a charged or aromatic amino acid; X₁₂₅ is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X₁₂₆ is any amino acid, but 10 preferably a small amino acid (Formula 10; Group 6 motif).

In one embodiment, peptides comprising a preferred amino acid sequence FYX₃ WF (SEQ ID NO: 1544) (Formula 1; Group 1; A6 motif) have been identified which competitively bind to sites on IR. Surprisingly, peptides comprising an amino acid sequence FYX₃ WF (SEQ ID NO:1544) 15 can possess agonist or antagonist activity at IR.

This invention also identifies at least two distinct binding sites on IR based on the differing ability of certain of the peptides to compete with one another and insulin for binding to IR. Accordingly, this invention provides amino acid sequences that bind specifically to one or both sites of IR. 20 Furthermore, specific amino acid sequences are provided which have either agonist or antagonist characteristics based on their ability to bind to the specific sites of IR.

In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R (e.g., Site 1 or Site 2) are 25 covalently linked together to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences are covalently bound together to form homo- or heterocomplexes.

In various aspects of the invention, monomer subunits are covalently 30 linked at their N-termini or C-termini to form N-N, C-C, N-C, or C-N linked dimer peptides. In one example, dimer peptides are used to form receptor

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complexes bound through the same corresponding sites, e.g., Site 1-Site 1 or Site 2-Site 2 dimers. Alternatively, heterodimer peptides are used to bind to different sites on one receptor or to cause receptor complexing through different sites, e.g., Site 1-Site 2 or Site 2-Site 1 dimers. In one novel aspect 5 of the invention, Site 2-Site 1 dimers find use as insulin agonists, while certain Site 1-Site 2 dimers find use as insulin antagonists.

In various embodiments, insulin agonists comprise Site 1-Site 1 dimer peptide sequences S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418; whereas other insulin agonists 10 comprise Site 2-Site 1 dimer peptide sequences S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520, as described herein below. In one preferred embodiment, an insulin agonist comprises the sequence of the S519 dimer peptide, which shows insulin-like activity in both *in vitro* and *in vivo* assays.

15 The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin or IGF-1. Such compounds may act as antagonists or agonists of insulin or IGF-1 function in cell based assays.

This invention further provides kits for identifying compounds that 20 bind to IR and/or IGF-1R. Also provided are therapeutic compounds that bind the insulin receptor or the IGF-1 receptor.

Other embodiments of this invention are the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells 25 which express the nucleic acids encoding the amino acid sequences which bind at IR and/or IGF-1R and possess agonist or antagonist activity.

This invention also provides amino acid sequences that bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR or IGF-1R.

30 This invention further provides specific amino acid sequences that possess agonist, partial agonist, or antagonist activity at either IR or IGF-1R.

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Such amino acid sequences are potentially useful as therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

5 In addition, the present invention provides structural information derived from the amino acid sequences of this invention, which may be used to construct other molecules possessing the desired activity at the relevant IR binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

10 Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 43A-43B, 44A-44B: Amino acid sequences identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio
15 Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R. HIT indicates binder; CAND indicates binder candidate; LDH indicates binding to lactate dehydrogenase (negative control); Sp/Irr indicates the ratio of specific binding over non-specific binding.

20 The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A
25 or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

Symbols in the listed sequences are: Q - TAG Stop; # - TAA Stop; * - TGA Stop; and ? - Unknown Amino Acid. It is believed that a W replaces the TGA Stop Codon when expressed. Except for the 20C and A6L
30 libraries, all libraries are designed with the short FLAG® Epitope DYKD

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(SEQ ID NO:1545; Hopp *et al.*, 1988, *BioTechnology* 6:1205-1210) at the N-terminus of the listed sequence and AAAGAP (SEQ ID NO:1546) at the C-terminus. The 20C and A6L libraries have the full length FLAG® epitope DYKDDDDK (SEQ ID NO:1547).

5 Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR (SEQ ID NOS:1-3).

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R (SEQ ID NOS:4-6).

10 Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR (SEQ ID NOS:7-29).

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R (SEQ ID NOS:30-33).

15 Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X₁₋₁₀NFYDW₁₈₋₂₁FVX₁₈₋₂₁ (SEQ ID NO:34; also referred to as "A6S") panned against IR (SEQ ID NOS:35-98).

Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X₁₋₁₀NFYDW₁₈₋₂₁FVX₁₈₋₂₁ (SEQ ID NO:34; also referred to as "A6S") panned against IGF-1R (SEQ ID NOS:99-166).

20 Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO: 167)) panned against IR (SEQ ID NOS:168-216).

25 Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO: 167)) panned against IGF-1R (SEQ ID NOS:217-244).

30 Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO: 245) (as indicated) panned against IR (SEQ ID NOS:246-305).

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Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO: 245) (as indicated) panned against IGF-1R (SEQ ID NOS:306-342).

5 Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHENFYDW₁₈₋₂₁FVRQVSX₂₁₋₂₆ (SEQ ID NO:343; H2C-A) panned against IR (SEQ ID NOS:344-430).

10 Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHENFYDW₁₈₋₂₁FVRQVSX₂₁₋₂₆ (SEQ ID NO:343; H2C-A) panned against IGF-1R (SEQ ID NOS:431-467).

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHXXFYXWFX₁₆₋₂₁ (SEQ ID NO:468; H2C-B) and panned against IR (SEQ ID NOS:469-575).

15 Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHXXFYXWFX₁₆₋₂₁ (SEQ ID NO:468; H2C-B) and panned against IGF-1R (SEQ ID NOS:576-657).

Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR (SEQ ID NOS:658-712).

20 Figure 2A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR (SEQ ID NO:713).

Figure 2B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IR (SEQ ID NOS:714-796).

25 Figure 2C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IGF-1R (SEQ ID NOS:797-811).

30 Figure 2D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:

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713)as indicated (20% dope; referred to as "F820") panned against IR (SEQ ID NOS:812-861).

Figure 2E: Formula 4 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS:862-925).

5 Figure 3A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR (SEQ ID NOS:926-928).

Figure 3B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (15% dope; referred to as "D815") panned against IR (SEQ ID 10 NOS:930-967).

Figure 3C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (20% dope; referred to as "D820") panned against IR (SEQ ID NOS:968-1010).

15 Figure 3D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (20% dope; referred to as "D820") panned against IGF-1R (SEQ ID NOS:1011-1059).

Figure 3E: Formula 6 motif peptide sequences identified from other 20 libraries panned against IR (SEQ ID NOS:1060-1061).

Figure 4A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R (SEQ ID NOS:1062-1077).

Figure 4B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR (SEQ ID NOS:1078-1082).

25 Figure 4C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR (SEQ ID NOS:1083-1086).

Figure 4D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR (SEQ ID NOS:1087-1088).

Figure 4E: Miscellaneous peptide sequences identified from a 30 random 20mer library panned against IGF-1R (SEQ ID NOS:1089-1092).

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Figure 4F: Miscellaneous peptide sequences identified from an X₁₄C X₆₋₂₀ library and panned against IGF-1R (SEQ ID NOS:1093-1113).

Figure 4G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide(SEQ ID NO: 1114) 5 as indicated (F815) panned against IGF-1R (SEQ ID NOS:1115-1118).

Figure 4H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO: 1119) as indicated (referred to as "NNKH") panned against IR (SEQ ID NOS:1120-1142).

10 Figure 4I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO: 1119) as indicated (referred to as "NNKH") panned against IGF-1R (SEQ ID NOS:1143-1154).

15 Figure 5A: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1155-1180).

Figure 5B: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1181-1220).

Figure 6: Illustration of 2 binding site domains on IR based on competition data.

20 Figure 7: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

Figure 8: Biopanning results and sequence alignments of Group 1 of IR-binding peptides (SEQ ID NOS:1221-1243). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: +++, >30X over background; ++, 15-30X; +, 5-15X; +, 2-5X; and 0, <2X.

Figures 9A-9B: Biopanning results and sequence alignments of Groups 2, 6, and 7 of IR-binding peptides (SEQ ID NOS:1244-1261). The 30 number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor.

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Absorbance signals are indicated by: +++, >30X over background; ++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

- Figures 10A-10C: Insulin competition data determined for various monomer and dimer peptides. Figure 10A shows the competition curve.
5 Figure 10B shows the symbol key for the peptides. Figure 10C shows the description of the peptides.

- Figures 11A-11D: Insulin competition data determined for various monomer and dimer peptides. Figure 11A shows the competition curve. Figure 11B shows the symbol key for the peptides. Figure 11C shows the
10 description of the peptides. Figure 11D shows IR binding affinity for the peptides.

- Figures 12A-12D: Results of free fat cell assays for truncated synthetic RP9 monomer peptides, S390 and S394. Figure 12A shows the results for peptide S390. Figure 12B shows the results for peptide S394.
15 Figure 12C shows the amino acid sequence of peptides S390 and S394 (SEQ ID NOS:1794 and 1788, respectively in order of appearance). Figure 12D shows the results for full-length RP9 peptide.

- Figures 13A-13C: Results of free fat cell assays for truncated synthetic RP9 dimer peptides, S415 and S417. Figure 13A shows the results for peptide S415. Figure 13B shows the results for peptide S417. Figure 13C shows the amino acid sequence of peptides S415 and S417 (SEQ ID NOS:1795-1796).

- Figures 14A-14C: Results of free fat cell assays for RP9 homodimer peptides, 521 and 535. Figure 14A shows the results for peptide 521. Figure 14B shows the results for peptide 535. Figure 14C shows the amino acid sequence of peptides 521 and 535.
25

- Figures 15A-15C: Results of free fat cell assays for RP9-D8 heterodimer peptides, 537 and 538. Figure 15A shows the results for peptide 537. Figure 15B shows the results for peptide 538. Figure 15C
30 shows the amino acid sequence of peptides 537 and 538.

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Figures 16A-16C: Results of free fat cell assays for RP9-D8 heterodimer peptides 537 and 538. Figure 16A shows the results for peptide 537. Figure 16B shows the results for peptide 538. Figure 16C shows the amino acid sequence of peptides 537 and 538.

5 Figures 17A-17B: Results of free fat cell assays for D8-RP9 heterodimer peptide, 539. Figure 17A shows the results for peptide 539. Figure 17B shows the amino acid sequence of peptide 539.

10 Figures 18A-18D: Results of free fat cell assays for Site 1/Site 2 dimer peptides with constituent monomer peptides with Site 1-Site 2 C-N (Figure 18A), Site 1-Site 2, N-N (Figure 18B), Site 1-Site 2, C-C (Figure 18C), and Site 2-Site 1, C-N (Figure 18D) orientations and linkages, respectively.

15 Figures 19A-19B: Results of human insulin receptor kinase assays for various monomer and dimer peptides. Figure 19A shows the substrate phosphorylation curve. Figure 19B shows the EC₅₀ values.

Figures 20A-20B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 dimer peptides. Figure 20A shows the substrate phosphorylation curve. Figure 20B shows the EC₅₀ values.

20 Figures 21A-21B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 peptides. Figure 21A shows the substrate phosphorylation curve. Figure 21B shows the EC₅₀ values.

25 Figures 22A-22B: Results of time-resolved fluorescence resonance transfer assays for assessing the ability of various monomer and dimer peptides to compete with biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figure 22A shows the binding curve. Figure 22B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

30 Figures 23A-23C: Results of time-resolved fluorescence resonance transfer assays indicating the ability of various monomer and dimer peptide

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to compete with biotinylated S175 monomer peptide or biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figures 23A-23B show the binding curves. Figure 23C shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 24A-24B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptide to compete with fluorescein labeled RP9 monomer peptide for binding to soluble human insulin receptor ectodomain. Figure 24A shows the binding curve. Figure 24B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560 and 2001-2002, respectively in order of appearance).

Figures 25A-25B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled RP9 monomer peptide for binding to soluble human insulin mini-receptor. Figure 25A shows the binding curve. Figure 25B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 26A-26B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin receptor ectodomain. Figure 26A shows the binding curve. Figure 26B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 27A-27B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin mini-

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receptor. Figure 27A shows the binding curve. Figure 27B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

5 Figure 28: A schematic drawing for the construction of protein fusions of the maltose binding protein.

Figure 29: BIACore analysis of competition binding between IR and maltose binding protein fusion peptides H2C-9aa-H2C, H2C, and H2C-3aa-H2C.

10 Figure 30: Stimulation of IR autophosphorylation *in vivo* by maltose binding protein fusion peptides.

Figures 31A-31C: Results of free fat cell assays for insulin and Site 2-Site 1 peptides, S519 and S520. Figure 31A shows the results for S519. Figure 31B shows the results for S520. Figure 31C shows the EC₅₀ values.

15 Figure 32A-32B: Results of human insulin receptor kinase assays for insulin and Site 2-Site 1 peptides S519 and S520. Figure 32A shows the substrate phosphorylation curve. Figure 32B shows the calculated Bestfit values.

Figure 33: Results of *in vivo* experiments showing the effect of 20 intravenous administration of Site 2-Site 1 peptide S519 in Wistar rats:

Figures 34A-34E: Results of phage competition studies with IGF-1 surrogates RP9 (Site 1) and D815 (Site 2) peptides. Phage: RP9 (A6-like); RP6 (B6-like); D8B12 (Site 2); and D815 (Site 2); Peptides: RP9 and D815. Figures 34A-34B show the competition curves. Figures 34C-34E show the symbol keys and peptide groups.

Figure 35A-35E: Phage competition studies with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Phage: RP9, RP6, D8B12, and D815; Peptides: D815-6L-RP9 and D815-12L-RP9. Figures 35A-35B show the competition curves. Figures 35C-35E show the symbol keys and peptide groups.

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Figure 36: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the agonist assay.

Figure 37: Results of IGF-1 antagonist assay using FDCP-2 cells.
5 Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the antagonist assay.

Figure 38: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides 20E2, S175, and RP9 were tested in the agonist assay.

Figures 39: Results of agonist and antagonist studies with surrogate
10 monomers and dimers. Monomers: D815 and RP9; Dimers: D815-6aa-
RP9 and D815-12aa-RP9.

Figure 40: Results of agonist and antagonist studies with surrogate monomers and dimers. Monomers: G33 and D815; Dimer: D815-6aa-G33.

Figure 41: Results of agonist and antagonist studies with surrogate
15 peptides and dimers. Monomers: G33, D815 and RP9; Dimers: D815-6aa-
RP9 and D815-12aa-RP9.

Figure 42: IGF-1 standard curve using FDCP-2 cells.

Figures 43A-43B: Peptide monomers identified from G33 and RP6
secondary libraries panned against IGF-1R (SEQ ID NOS:1262-1432).
20 Figure 43A shows peptides from G33 secondary library; Figure 43B shows
peptides from RP6 secondary library.

Figures 44A-44B: Peptide dimers identified from libraries panned
against IR or IGF-1R (SEQ ID NOS:1433-1540). Figure 44A shows dimer
peptides panned against IR; Figure 44B shows dimer peptides panned
25 against IGF-1R.

Figure 45: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

30 Figure 46: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815 (rD815)

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on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 47: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate RP9 on the 5 binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 48: Results of heterogeneous time-resolved fluorometric assay showing the effect of recombinant peptide surrogate D815-6-G33 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to 10 recombinant human IGF-1R (rhIGF-1R).

Figure 49: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815-6-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

15 Figure 50: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815-12-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

20 Figure 51: Results of heterogeneous time-resolved fluorometric assays showing the effect of IGF-1 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

25 Figure 52: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of Site 1 peptide surrogates, Site 2 peptide surrogates, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

30 Figure 53: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of various peptide monomers and dimers on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

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Figure 54: Results of glucose uptake assays in SGBS cells showing the potency of peptide S597 relative to human insulin.

Figure 55: Results of glucose-lowering assays in rats showing the potency of peptide S557 and S597 relative to human insulin.

5 Figure 56: Results of glucose-lowering assays in fasted Goettingen minipigs showing the potency of peptide S597 relative to human insulin.

Figure 57: Results of studies of disappearance of I^{125} -labelled peptides from site of injection.

V. DETAILED DESCRIPTION OF THE INVENTION

10 This invention relates to amino acid sequences comprising motifs that bind to the insulin receptor (IR) and/or insulin-like growth factor receptor (IGF-1R). In addition to binding to IR and/or IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at IR or IGF-1R. In addition, the amino acid sequences bind to separate
15 binding sites (Sites 1 or 2) on IR or IGF-1R.

Although capable of binding to IR or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are not based on the native insulin or IGF-1 sequences, nor do they reflect an obvious homology to any such sequences.

20 The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this invention. Thus, any amino acid sequence comprising at least one of the IR
25 or IGF-1R binding motifs disclosed herein, and which binds to IR or IGF-1R is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF-1 agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e., non-naturally occurring, peptides, or polypeptides. Amino acid sequences useful
30 in the invention may be obtained through various means such as chemical

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synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR sufficient to provide adequate binding for the intended purpose.

5 Thus, for use as a therapeutic, the peptide, polypeptide, or protein provided by this invention should have an affinity (K_d) of between about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about 10^{-12} M. Most preferably, the affinity is 10^{-10} to about 10^{-12} M. For use as a reagent in a

10 competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

The present invention describes several different binding motifs, which bind to active sites on IR or IGF-1R. The binding motifs are defined

15 based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application

20 Serial No. 09/538,038, filed March 29, 2000.

Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, sequence tags (e.g., FLAG® tags) or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends) as described in detail herein. Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy

25 and amino terminal regions of the consensus motifs described below, which

30 comprise sequence tags (e.g., FLAG® tags), or which contain amino acid

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residues that are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) such as lysine which promote the stability or biotinylation of the amino acids sequences 5 may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

Peptides that bind to IR or IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley *et al.*, 10 International Application PCT/US00/08528 filed March 29, 2000 and Beasley *et al.*, U.S. Application Serial No. 09/538,038 filed March 29, 2000, which are incorporated by reference in their entirety.

A. Consensus Motifs

The following motifs have been identified as conferring binding 15 activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1; Group 1; the A6 motif) wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be any small polar amino acid, but is preferably selected from aspartic acid, 20 glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF (SEQ ID NO:1554) and FYEWF (SEQ ID NO:1555). The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist 25 activity at IR depending on the identity of amino acids flanking A6. See Figure 5A.

Amino acid sequences that comprise the A6 motif and possess agonist activity at IR, include but are not limited to, D117/H2C: FHENFYDWFVRQVSKK (SEQ ID NO:1556); D117/H2 minus terminal 30 lysines: FHENFYDWFVRQVS (SEQ ID NO:1557); RP9:

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GSLDESFYDWFERQLGKK (SEQ ID NO:1558); RP9 minus terminal lysines: GSLDESFYDWFERQLG (SEQ ID NO:1559); and S175: GRVDWLQRNANFYDWFWVAELG (SEQ ID NO:1560). Preferred RP9 sequences include GLADEDFYEWFERQLR (SEQ ID NO:1561),
5 GLADEFYEWFDRQLS (SEQ ID NO:1562), GQLDEDFYEWFDRQLS (SEQ ID NO:1563), GQLDEDFYAWFDRQLS (SEQ ID NO:1564), GFMDESFYEWFERQLR (SEQ ID NO:1565), GFWDESFYAWFERQLR (SEQ ID NO:1566), GFMDESFYAWFERQLR (SEQ ID NO:1567), and GFWDESFYEWFERQLR (SEQ ID NO:1568). Nonlimiting examples of
10 Group 1 (Formula 1; A6) amino acid sequences are shown in Figures 1A-1O.

2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, Group 3; the B6 motif) wherein X_6 and X_7 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_6 is phenylalanine and X_7 is tyrosine. X_8 , X_9 , X_{11} , and X_{12} may be any amino acid. X_{10} and X_{13} are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X_{10} is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X_{13} is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist
15 at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are FYX₈X₉LX₁₁X₁₂L (SEQ ID NO:1569), F Y X₈X₉I X₁₁X₁₂L (SEQ ID NO:1570), F Y X₈A I X₁₁X₁₂L (SEQ ID NO:1571), and F
20 Y X₈Y F X₁₁X₁₂L (SEQ ID NO:1572).

Another Formula 2 motif for use with this invention comprises F Y X₈Y F X₁₁X₁₂L (SEQ ID NO:1573) and is shown as Formula 2A ("NNRP") below: X₁₁₅X₁₁₆X₁₁₇X₁₁₈F Y X₈Y F X₁₁X₁₂LX₁₁₉X₁₂₀X₁₂₁X₁₂₂, (SEQ ID NO:1574) wherein X₁₁₅-X₁₁₈ and X₁₁₈-X₁₂₂ may be any amino acid which allows for binding to IR or IGF-1R. X₁₁₅ is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid, and arginine.
25 Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its
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presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

- 5 X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are preferably glycine, aspartic acid, glutamic acid, asparagine, or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or 10 alanine. X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine. X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid. X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most 15 preferably aspartic acid. X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid. X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid. X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or 20 tyrosine. X_{122} is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid. Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.
- 25 3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14} , X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15} , X_{16} , X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic 30 amino acid, but preferably phenylalanine or tyrosine, and most preferably

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phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at X₁₈.

4. $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ (Formula 4; Group 7; the F8 motif) wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid. X₃₅ and X₃₇ may be any amino acid when the F8 motif is used as an IR binding ligand or as a component of an IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at X₃₇ and a hydrophobic amino acid, particularly, leucine, is preferred at X₃₅. X₂₃ is a hydrophobic amino acid.
- 5 10 15 20 25 30 Methionine, valine, leucine or isoleucine are preferred amino acids for X₂₃, however, leucine which is most preferred for preparation of an IGF-1R binding ligand is especially preferred for preparation of an IR binding ligand. At least one cysteine is located at X₂₄ through X₂₇, and one at X₃₉ or X₄₀. Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions X₂₄ and X₃₉ if the cysteines occupy other positions.
- 20 In one embodiment, for example, the cysteine at position X₂₄ may occur at position X₂₇ which will produce a smaller loop provided that the cysteine is maintained at position X₃₉. These smaller looped peptides are described herein as Formula 5, infra. X₂₇ is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position X₂₇ decreases binding to IR but has less of an effect on binding to IGF-1R. X₃₁ is any aromatic amino acid and X₃₂ is any small amino acid. For binding to IGF-1R, glycine or serine is preferred at position X₃₁, however, tryptophan is highly preferred for binding to IR. At position X₃₂, glycine is preferred for both IGF-1R and IR binding. X₃₆ is an aromatic amino acid. A preferred consensus sequence for F8 is X₂₂ LC X₂₅ X₂₆ E X₂₈ X₂₉ X₃₀ WG X₃₃ X₃₄ X₃₅

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X₃₆ X₃₇ X₃₈ C X₄₀ X₄₁ (SEQ ID NO:1575) whereas the amino acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"; SEQ ID NO:1576). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over 5 IGF-1R. Figures 2A-2E list nonlimiting examples of Formula 4 amino acid sequences.

5. X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆ X₅₇ X₅₈ X₅₉ X₆₀ X₆₁ (Formula 5; mini F8 motif) wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid. X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are 10 hydrophobic amino acids, however, X₄₃ and X₄₆ are preferably leucine, whereas X₅₀ is preferably phenylalanine or tyrosine but most preferably phenylalanine. X₄₇ and X₅₉ are cysteines. X₄₈ is preferably a polar amino acid, i.e., aspartic acid or glutamic acid, but most preferably glutamic acid. Use of the small amino acid at position 54 may confer IGF-1R specificity. 15 X₅₁, X₅₂, and X₅₇ are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is X₄₂ X₄₃ X₄₄ X₄₅ L C E X₄₉ F G G X₅₃ X₅₄ X₅₅ X₅₆ G X₅₈ C X₆₀ X₆₁ (SEQ ID NO:1577). Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR.

6. X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ (Formula 6; Group 2; the D8 motif) wherein X₆₂, X₆₅, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ may be any amino acid. X₆₆ may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of X₆₆ with glutamine or valine may result in attenuation of binding. X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids. X₆₃ is preferably 20 leucine, isoleucine, methionine, or valine, but most preferably leucine. X₇₀ and X₇₄ are preferably valine, isoleucine, leucine, or methionine. X₇₄ is most preferably valine. X₆₄ is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. X₆₇ and X₇₅ are aromatic amino acids. Whereas tryptophan is highly preferred at X₆₇, X₇₅ is preferably 25 tyrosine or tryptophan but most preferably tyrosine. X₇₂ and X₇₉ are 30

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cysteines that again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence.

D8 is most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is X₆₂ L X₆₄ X₆₅ X₆₆ W X₆₈ X₆₉ X₇₀ X₇₁ C X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ C X₈₀ X₈₁ (SEQ ID NO:1578). Examples of specific peptide sequences comprising this motif include D8: KWLDQEWAQVQCEVYGRGCPSSK (SEQ ID NO:1579); and D8 minus terminal lysines: KWLDQEWAQVQCEVYGRGCP (SEQ ID NO:1580). Preferred D8 monomer sequences include SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581) and SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582). Preferred D8 dimer sequences include SLEEEWAQIECEVYGRGCP (SEQ ID NO:1583), and SLEEEWAQIECEVWGRGCP (SEQ ID NO:1584). Nonlimiting examples of Group 2 (Formula 6; D8) amino acid sequences are shown in Figures 3A-3E.

7. H X₈₂, X₈₃, X₈₄ X₈₅ X₈₆ X₈₇ X₈₈ X₈₉ X₉₀ X₉₁ X₉₂ (Formula 7) wherein X₈₂ is proline or alanine but most preferably proline; X₈₃ is a small amino acid more preferably proline, serine or threonine and most preferably proline; X₈₄ is selected from leucine, serine or threonine but most preferably leucine; X₈₅ is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X₈₆ may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X₈₇ is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid X₈₈, X₈₉ and X₉₀ may be any amino acids; X₉₁ is an aliphatic amino acid with a strong preference for leucine as is X₉₂. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is H P P L S X₈₆ L X₈₈ X₈₉ X₉₀ L (SEQ ID NO:1585). The Formula 7 motif binds to IR with little or no binding to IGF-1R.

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8. Another sequence is $X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111}$ $X_{112} X_{113} X_{114}$ (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X_{106} through X_{111} are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end. Accordingly, the most preferred sequence for $X_{107} X_{108} X_{109} X_{110} X_{111}$ is WPTYW (SEQ ID NO:1586). At least one of the three amino acids on the amino terminal ($X_{104}, X_{105} X_{106}$) and at least one of the amino acids carboxy terminal ($X_{112} X_{113} X_{114}$) ends immediately flanking X_{107} - X_{111} are preferably a cysteine residue, most preferably at X_{105} and X_{113} respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X_{104} and X_{114} are both small amino acids such as, for example, alanine and glycine. Most preferably, X_{104} is alanine and X_{114} is glycine. X_{105} may be any amino acid but is preferably valine. X_{112} is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG (SEQ ID NO:1587).

9. An amino acid sequence comprising JBA5: $DYKDLCQSWGVRIGWLAGLCPKK$ (SEQ ID NO:1541); or JBA5 without terminal lysines: $LCQSWGVRIGWLAGLCP$ (SEQ ID NO:1542) (Formula 9). The Formula 9 motif is another motif believed to form a cysteine loop that possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.

10. $W X_{123} G Y X_{124} W X_{125} X_{126}$ (SEQ ID NO:1543) (Formula 10; Group 6) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. In one embodiment of

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- the present invention, the Formula 10, Group 6 motif is WPGY (SEQ ID NO: 1588). Examples of specific peptide sequences comprising this motif include E8: KVRGFQGGTVWPGYEWLRNAKK (SEQ ID NO:1589); and E8 minus terminal lysines: KVRGFQGGTVWPGYEWLRNAA (SEQ ID NO:1590). Preferred Group 6 sequences include WAGYEWLF (SEQ ID NO:1591), WEGYEWL (SEQ ID NO:1592), WAGYEWL (SEQ ID NO:1593), WEGYEWLF (SEQ ID NO:1594), and DSDWAGYEWFEEQLD (SEQ ID NO:1595). Nonlimiting examples of Group 6 amino acid sequences are shown in Figures 4A-4B.
- 10 The IR and IGF-1R binding activities of representative Group 1 (Formula 1; A6); Group 2 (Formula 6; D8); and Group 6 (Formula 10); and Group 7 (Formula 4; F8) amino acid sequences are summarized in Figures 8 and 9A-9B. Group 1 (Formula 1; A6) amino acid sequences contain the consensus sequence FyxWF (SEQ ID NO:1596), which is typically agonistic in cell-based assays. Group 2 (Formula 6; D8) amino acid sequences are composed of two internal sequences having a consensus sequence VYGR (SEQ ID NO:1597) and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin. Group 7 (Formula 4; F8) amino acid sequences are composed of two internal exemplary sequences which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

B. Amino And Carboxyl Terminal Extensions Modulate Activity of Motifs

- In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the

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possible use of the motifs with other substitutions, additions or deletions that allow for binding to IR, IGF-1R, or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino 5 terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 X₉₃ X₉₄ X₉₅ X₉₆ X₉₇ wherein X₉₃ may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X₉₄ and X₉₇ 10 are any amino acid; X₉₅ is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X₉₅ however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X₉₅ may indicate that these amino acids should be avoided to maintain or enhance 15 sufficient binding to IR and IGF-1R. X₉₆ is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X₉₆ may be used to enhance IR selectivity.

2. Formula 2

20 B6 with amino terminal and carboxy terminal extensions may be represented as X₉₈ X₉₉ B6 X₁₀₀. X₉₈ is optionally aspartic acid and X₉₉ is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X₉₈ and a proline at X₉₉ is associated with an enhancement of binding for both IR and 25 IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X₁₀₀, an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

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3. Formula 3

An amino terminal extension of Formula 3 defined as $X_{101} X_{102} X_{103}$ revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, 5 more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

4. Formula 10

In one preferred embodiment, Formula 10 sequences W X_{123} G Y
10 X_{124} W X_{125} X_{126} (SEQ ID NO:1543) can include an amino terminal extension comprising the sequence DSD and/or a carboxy terminal extension comprising the sequence EQLD (SEQ ID NO:1598).

C. IR Binding Preferences

As indicated above, the amino acid sequences containing the motifs 15 of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non- 20 cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in 25 Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 44A-44B. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

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IGF-1R-SELECTIVE SEQUENCES

TABLE 1

FORMULA 1 (Group 1; A6-like):

Comparisons

Ratios over Background

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
A6L-0-E6-IR	1599	YRGMLVLAGSSDAGKVAFFPARIGQTVAVNNDWVF	31.0	31.0	1.8	17.0	0.1
H2CA-4-G9-IGFR	1600	GIIQSCLPESFYDWPAQVSDPMWCW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	1601	VGRASGFPEFYDWFGRLSLSQSGEQ	4.9	10.5	0.7	14.6	0.1
A6L-0-E4-IR	1602	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNNDWVF	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	1603	YRGMLVLGRISGAGKAASERPARIGQKVSAVNNDWVF	27.0	26.0	2.0	13.0	0.1
H2CA-4-F5-IGFR	1604	VGYGGQGDENFYDWIFIRQVSGLGQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	1605	SACQFDCHENFYDWPARQVSGGAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	1606	SAAQLEFFQESFYDWFLRQVAESSPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	1607	AVRATRFDEAFYDWVFRQILSDGCNPK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	1608	VNQSGSIHENFYDWFERQVSHORGVR	4.9	5.7	1.0	5.9	0.2
H2CA-4-A3-IGFR	1609	APDPSDFQEIPDYDWFRQVSRMPCGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	1610	SSCDGAGHESSTEWFRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	1611	RAGSSDDEFHEFYEWFRQVSSLKIK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H9-IGFR	1612	QAVQPGFHEBEFYDWFRQVSGVGGG	3.9	4.1	1.0	4.2	0.2
E4Dq-4-H2-IR	1613	GFRREGNFYEWFRQQAQTV	37.8	33.9	8.2	4.1	0.2
H2CA-4-F7-IGFR	1614	SS1GGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	1615	QSPVGSSSHEDFYDWFFRQVAQSGCAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D1-IGFR	1616	NYRRQVFGNMFYDWFDROVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	1617	TLDGGSFPEFYDWFRQLSYTRNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	1618	FYVQQWGHENFYDWFRQVSQSGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	1619	LRRQAPVFEENFYDWFRQVSQGDGVG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	1620	RCGRELVHSIFYDWFRQVAGRTCP S	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	1621	CCLLCRFFQONYDWFCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B5-IGFR	1622	PPLASDLDIVFYGMFWQQVSPPERGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	1623	GAPVDQHLHEDFYDWFRQVSQATG	4.1	3.4	1.0	3.5	0.3
E4Dq-2-D11-IR	1624	GPREGFSFYDWFOAQVT	40.2	11.1	3.3	3.4	0.3
20E2Bβ-4-C6-IR	1625	SQGSAFYAWFDQVLRTVHSA	22.4	6.2	1.9	3.3	0.3
H2CA-4-H9-IGFR	1626	RGAVAGFFHDQFYDWFRQVSRYHKFG	8.7	5.6	1.9	3.0	0.3

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Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
H2CA-2-B11-IGFR	1627	AICDAGPFIEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	1628	LGYOEPFQPNFYDWFVHQVSGAENAG	13.2	6.3	2.2	2.9	0.3
A6S-2-D11-IR	1629	EAASLGSQDRNFDYDWFVHQV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	1630	TEVASSDQDGNFYDWFVHQVLR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	1631	TSEVQRSSQDNFYDWFVHQVQA	33.1	24.7	9.8	2.5	0.4
H2CA-3-B11-IGFR	1632	HLADQFHEKFKYDWFERQISSRCND	4.7	2.2	1.0	2.2	0.5
H2CA-3-C11-IGFR	1633	FRTLAAQDSDSFYDWFDRQVSQGAAGER	9.3	3.3	1.6	2.1	0.5
A6-PD1-IGFR	1634	SFHEDPYDWFDRQVSGLKK					
H2C-PD1-IGFR (RP9)	1558	GSLDESFTDWEERQLGKK					

FORMULA 2 (Group 2; B6-like):

Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	1635	TFYSCSLLTGTPOPNRGWPWCR	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	1636	FFYDCLLALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
B6Ha-1-B5-IR	1637	CCTTEMVMDARDDPFYHKLSLUVTG	41.5	20.5	1.0	20.5	0.0
R20B-4-A6-IR	1638	RGQSDAFVSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1
20E2B-1-A6-IGFR	1639	GVRAMSFTDALVSVLGLGPSSG	18.6	18.1	1.1	16.8	0.1
R20a-4-20A12-IR	1640	RLFYCGGIQALGANLYGGCV	48.6	39.9	2.4	16.6	0.1
20E2Bβ-4-G7-IR	1641	LQPCSGFTFECTRLIGVKLSG	19.9	25.2	1.6	15.8	0.1
NNRPY-4-B11-IR	1642	LKDGFYDFWQLHLGS	4.1	18.7	1.2	15.5	0.1
20E2B-3-C6-IGFR	1643	VEGRGLFTDLRROLLARRONG	17.9	16.8	1.1	14.8	0.1
B6Ha-1-A2-IR	1644	RGCNDDGGKIGKNSDDPFYHKLSLICGG	22.3	14.6	1.0	14.6	0.1
20E2A-4-F11-IGFR	1645	QGGSSASFYDADLRLMRIGG	21.3	18.8	1.3	14.6	0.1
B6Ha-3-E9-IR	1646	RCBEKQAEVGPSSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
20C-3-F6-IGFR	1647	DRDFCRFYFBRUTALVGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20E2B-4-H3-IGFR	1648	KLNHNLMPFTYGLQRUVWAGLIG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	1649	GNGDSGMFYQQLSLILVGRDMHV	13.1	8.9	0.6	13.8	0.1
20C-3-A1-IGFR	1650	SSYGDGFYMLPSLGLVVASQELC	26.5	20.8	1.5	13.7	0.1
20E2B-3-E3-IGFR	1651	PDLHKGFYAGLAQLIRGOLLIS	22.4	16.3	1.3	13.1	0.1
R20a-3-20E2-IR	1652	FYDAIDQLVRSARAGGTRD	46.3	39.9	3.1	12.9	0.1
20E2B-4-H12-IGFR	1653	YSCGDGFYSLSLQDGGQFRC	6.5	9.7	0.8	12.8	0.1
B6Ha-3-F11-IR	1654	RGMKEEVLVGEGSTDPFYHKLSLQGS	49.5	18.7	1.6	11.7	0.1

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Ratios over Background

Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
B6Hg-3-E8-IR	1689	'RGKTAAVIVGRPADPFYHKLSELLOGG	47.6	5.3	1.1	4.8	0.2
B6Hg-3-F10-IR	1690	GCVIEWQKTHGASDPFYHKLSELGGCS	47.2	8.8	1.9	4.6	0.2
B6Hg-2-D6-IR	1691	GRTMAVMAAGCPDPFYHKLSELVQGG	33.5	4.4	1.0	4.4	0.2
B6Hg-3-E7-IR	1692	GCAVVEEAERSRGDPFYHKLSEL1QGC	47.0	5.6	1.3	4.3	0.2
B6Hg-2-D1-IR	1693	GCEVIVEEGDSADPFYHKLSEL0QGS	11.7	5.4	1.3	4.2	0.2
20E2A-3-D10-IGFR	1694	MNVVDGFYDALHQLVVAQSLSG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	1695	LSVALSFIDALGQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
B6Hg-4-G8-IR	1696	GGTKAVAKV/GTRDDPFYHKLSEL1QGS	32.3	6.1	1.7	3.6	0.3
B6L-4-D7-IR	1697	ABTSVQVGWIPRLQSVPWGPGEHNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6Hg-1-A3-IR	1698	SRAKVEAEMPDSGDPFYHKLSEL1ASG	37.4	2.6	0.8	3.3	0.3
B6Hg-3-F7-IR	1699	SRVAATKEKRPSDDPFYHKLSEL1QGS	41.5	3.1	1.0	3.1	0.3
B6Hg-2-D8-IR	1700	SSETAKMVGTRDDPFYHKLSEL1VQGS	19.3	3.0	1.0	3.0	0.3
B6Hg-1-B3-IR	1701	GCITANGAGDGPFYHKLSELGGCS	33.1	3.2	1.1	2.9	0.3
B6Hg-3-E5-IR	1702	RCGDEGMQENRRDGPFYHKLSELFGGC	28.8	2.9	1.0	2.9	0.3
20E2A-4-G11-IGFR	1703	MNVVFISFYDALDQLVCQRIGC	20.7	3.3	1.3	2.6	0.4
20E2B0-3-C7-IR	1704	QSGSGDFDYLISRLLIRGNGDG	1.5	3.1	1.5	2.0	0.5
B6Hg-3-E6-IR	1705	CGAKMTGTPADPFYHKLSELL0RG	18.2	2.3	1.2	1.9	0.5
20E2A-3-A3-IGFR	1706	GHYFGFSFYDALDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
B6L-4-A7-IR	1707	AGTPAQVG-NRLWSWPGPGEHNTVDPFYHKLSEL1RESGA	11.6	3.4	1.9	1.8	0.6
B6Hg-3-F1-IR	1708	CSMAAVAEKGDDDDPFYHKLSEL1CQGS	22.5	2.4	1.3	1.8	0.5
B6L-3-G6-IR	1709	VDTPAQVGNNRLWSWPGPGEHNTVDPFYHKLSEL1RESGA	7.6	2.5	1.8	1.4	0.7
B6L-3-G5-IR	1710	AETSAQVGWQBLWSWPGPDIHSTLDPFYHKLSEL1RESGA	11.5	2.0	1.4	1.4	0.7
20E2A-3-A4-IGFR	1711	AGSVTISFYDAMEQLVATGTSA	16.8	2.5	1.8	1.4	0.7
B6-PD1-IGFR	1712	TDDGFYDALEOLQVQGSKK					
20E2-PD1-IGFR (RP:0)	1713	GSFYEAQLOQVQGSKK					

FORMULA 10 (Group 6):

Ratios over Background

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
R20B-4-E8-IR	1714	VRGFQGGTWWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1
40F-4-D1-IGFR	1715	LSCLAYSREGIWRPSTDLGLGRSVGECSVSPTRWGYDMFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	1716	GLDHSDAVGTVHLGFAWPQAQRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	1717	W.GYAWLS	4.9	4.5	0.4	11.7	0.1

Comparisons

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Ratios over Background

Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20E2B-3-D2-IGFR	1655	IQQELITFYDILLHRLVSELGSL	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	1656	GGTEVDYRALERLVRGSQL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	1657	LRIANLFYQRLMDLARQGGG	15.7	16.7	1.5	11.1	0.1
B6Hg-2-C4-IR	1658	RCGRN*AEHGAGDDPFTHKLSLVC	20.7	9.9	0.9	11.0	0.1
R20e-4-20C11-IR	1659	DRAFYNGLRLDVGAIVGAWD	43.7	30.8	3.0	10.3	0.1
20E2B-4-F8-IGFR	1660	PVGYQGFYEGLSRLVLRGRGGW	12.3	7.3	0.8	9.7	0.1
20E2B-1-A11-IGFR	1661	RFSTDTGFYQYLLALWGGPGV	15.0	9.5	1.0	9.7	0.1
20E2B-3-DA-IGFR	1662	NSRDGGFYQLLERLJGFPTVG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	1663	VVTPVNFYRALEALVRG_RLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	1664	QPAPPDFYSALMKLIGRGGS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	1665	PGTDLGQFYTQALRCVWLGQACD	11.7	4.9	0.6	8.1	0.1
20E2B-4-F10-IGFR	1666	AQPCCGFYGLLEQLVRSVCD	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	1667	QPDHSYFYSLLQELVGSSEERL	11.9	14.7	1.9	7.7	0.1
20C-3-A4-IGFR	1668	QFYGGCLLDLSLGVPSPWRRCITA	17.7	8.8	1.2	7.6	0.1
20E2B-3-D11-IGFR	1669	LGVTDGFYTAALGYLIGLICVGQF	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	1670	CMM_DGFYAGLGCLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	1671	ICTGQFQVLGCLLIGTISAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	1672	QGNVLDFGWIGRLLAKQSD	10.3	6.2	0.9	7.3	0.1
20E2B-3-E12-IGFR	1673	VATSGQFYSGLSLLQGGGNV	13.9	6.0	0.8	7.3	0.1
20E2B-2-B8-IGFR	1674	IWATGDDPYRLSQLVMGRVT	17.4	5.7	0.8	7.2	0.1
NNRPY-4-A9-IR	1675	EGSGFYGFYFFSLGLQG	3.0	10.0	1.4	7.1	0.1
20E2B-4-G11-IGFR	1676	ROGTTGSEYMLBOLLYGARGP	8.9	4.5	0.6	7.0	0.1
20E2B-3-D6-IGFR	1677	DSVGDNFYYQLLBSLYGGHVG	20.7	17.8	2.6	6.9	0.1
B6Hg-2-C7-IR	1678	RGIVAMMBATEVGSDADPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1
20E2B-2-B7-IGFR	1679	LSSDGQPYRALNLILQGSAGR	18.0	6.1	0.9	6.7	0.1
20E2B-3-C4-IGFR	1680	ASSASGTYELLQRLAGLILEV	23.4	20.4	3.3	6.2	0.2
20C-3-E4-IGFR	1681	PFYRCRSLRLGGQGLGSRLGSCIGD	37.7	7.7	1.3	6.0	0.2
NNRPY-4-A1-IR	1682	LIGGGFYSYFNNSVRLIET	9.7	10.9	1.8	6.0	0.2
20E2B-4-H8-IGFR	1683	PAGPCGFTYCGLGLLHGDOSP	7.2	5.3	0.9	5.9	0.2
20E2B-4-H9-IGFR	1684	RCQGTGTYTCIQELIGCPDD	4.5	5.2	0.9	5.6	0.2
B6Hg-2-C10-IR	1685	SGAKVITVVTGDSGDPFHKLSELQGS	46.9	5.8	1.1	5.3	0.2
20E2A-3-C7-IGFR	1686	VGTVAQYDIAAQLVARASRV	17.6	5.4	1.1	5.1	0.2
20E2B-1-A8-IGFR	1687	TLRSPPTYDWLEMVLTHGQGG	16.1	4.4	0.9	5.0	0.2
NNRPY-4-A7-IR	1688	RFDPFTSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2

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Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence that is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R.

For example, A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X₉₅. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X₁₁. Tryptophan at X₁₃ also favors selectivity of IR. A tryptophan amino acid at X₁₃ rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X₁₅ favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X₂₃ is essentially required for IR binding. In addition, tryptophan at X₃₁ is also highly preferred. At X₃₂, glycine is preferred for IR selectivity.

D. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 6, competition data indicate that peptides comprising the A6 motifs compete for binding to the same site on IR (Site 1) whereas the D8 motifs compete for a second site (Site 2). The identification of peptides that bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 7.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

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TABLE 2

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF) (SEQ ID NO: 1596):			
5	Clone	Sequence	
	G3	KRGGGTFYEWFESALRKHGAGKK	
	H2	VTFTSAVFHENFYDWFWVRQVSKK	
	H2C	FHENFYDWFWVRQVSKK	
10	A6S-IR3-E12	GRVDWLQRNANFYDWFWVAELG	
	A6S-IR4-G1	NGVERAGTGDNFYDWFWVAQLH	
	H2CB-R3-B12	QSDSGTVHDRFYGWFRDTWAS	
	20E2A-R3-B11	GRFYGWFQDAIDQLMPWGFDL	
	rB6-F6	RYGRWGLAQQFYDWFWDR	
15	E4D α -1-B8-IR-	GFREGQRWYWFWVAQVT	
	H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	
	H2CB-R3-D2	WTDVDGFHSGFYRWFQNQWER	
	H2CB-R3-D12	VASGHVLHGQFYRWFVDQFAL	
	H2CB-R4-H5	QARVGNVHQQFYEWFWREVMQG	
20	H2C-B-E8*	TGHRLGLDEQFYWWFRDALSG	
	H2CB-3-B6-IR-	VGDFCVSHDCFYGWFLRESMQ	
	A6S-IR2-C1	RMYFSTGAPQNFYDWFWVQEWD	
B6-like (FYxxLxxL) (SEQ ID NO: 1732):			
25	Clone	Sequence	
	20C11	KDRAFYNGLRDLVGAIVGAWDKK	
	20E2	DYKDFYDAIDQLVRGSARAGGTRDKK	
	B62-R3-C7	EHWNNTVDPFYFTLFPEWLRESG	
	B62-R3-C10	EHWNNTVDPFYQYFSELLRESG	
30	20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWWWDQG	
	20E2-B-E3*	IQGWEPFYGWFFDDVVAQMFEQ	
	20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC	
	RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS	
	RPNN-4-F3-HOLO*	HYNAFYEYFQVLLAETW	
35	20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER	
	RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT	
	20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG	
	20E2-B-E12	GNSGGSFYRYFQLLSDGMS	
	20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC	
40	Reverse B6-like (LxxLxxYF) (SEQ ID NO: 1747):		
	Clone	Sequence	
	rB6-A12	LDALDRLMRYFEERPSL	
	rB6-F9	PLAELWAYFEHSEQGRSSAH	
45	rB6-4-E7-IR	LDPLDALLQYFWSPGH	
	rB6-4-F9-IR	RGRLGSLSTQFYNWFAE	
	rB6-E6	ADELEWLLDYFMHQPRP	
	rB6-4-F12-IR	DGVLEELFSYFSATVGP	
50	Group 6 (WPxYxWL) (SEQ ID NO: 1754):		
	Clone	Sequence	
	R20 β -4-A4-IR	WPGYLFFEEALQDWWRGSTED	
55	Peptides by design**:		
	Clone	Sequence	
	H2C-PD1-IR-	AAVHEQFYDWFWADQYKK	
	A6S-PD1-IR-	QAPSNFYDWFWREWDKK	
	20E2-PD1-IR-	QSFYDYIEELLGGEWKK	

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B6C-PD1-IR-

DPFYQGLWEWLRESGKK

1759

REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

5

F8-derived (Long C-C loop):

	Clone	Sequence	SEQ ID NO:
	F8	HLCVLEELFWGASLFGYCSG	1760
	F8-C12	FQSLLEELVWGAAPLFRYGTG	1761
10	F8-Des2	PLCVLEELFWGASLFGYCSG	1762
	F8-F12	PLCVLEELFWGASLFGQCSG	1763
	F8-B9	HLCVLEELFWGASLFGQCSG	1764
	F8-B12	DLRVLCLELFGGAYVLGYCSE	1765
15	NNKH-2B3	HRSVLKQLSWGASLFGQWAG	1766
	NNKH-2F9-	HLSVGEELSWWVALLGQWAR	1767
	NNKH-4H4-	APVSTEELRWGALLFGQWAG	1768

20

D8-derived (Small C-C loop):

	Clone	Sequence	SEQ ID NO:
	D8	KWL DQEWA WVQCEV YGRGCP SKK	1769
	D8-G1	Q LEE EWA G VQCEV YGR ECP S	1770
25	D8-B5-	A LEE EWA W VQVR SIRS GLPL	1771
	D8-A7	S LD QEW A W VQCEV YGR GCL S	1772
	D8-F1-	W LEHEWA QI Q CELY GRG CTY	1773

Midi C-C loop:

	Clone	Sequence	
30	D8-F10	GLEQGCPWVGLEVQCRGCP S	1774
	F8-B12-	DLRVLCLELFGGAYVLGYCSE	1775
	F8-A9	PLWGLC E LFGGASLFGYCSS	1776

35

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides; * Peptides synthesized and currently being purified; ~ Peptides planned.

- In various aspects of the present invention, amino acid sequences comprising Site 1 motifs may bind to Site 1 of IR or Site 1 of IGF-1R.
- 40 Similarly, amino acids sequences comprising Site 2 motifs may bind to Site 2 of IR or Site 2 of IGF-1R. However, specific peptides may show higher binding affinity for IR than for IGF-1R, while other peptides may show higher binding affinity for IGF-1R than for IR. In addition, Site 1 and Site 2 on IR do not "crosstalk", i.e., Site 1-binding sequences do not compete with Site 2-binding sequences at IR. In contrast, Site 1 and Site 2 on IGF-1R do show some crosstalk, suggesting an allosteric effect. These aspects are illustrated in the Examples described hereinbelow.

E. Multivalent Ligands

This invention provides ligands that preferentially bind different sites on IR and IGF-1R. The A6 amino acid sequence motif confers binding to IR at Site 1 (Figure 6). The D8 amino acid sequence motif confers binding to 5 IR at Site 2 (Figure 6). Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences that bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the 10 same corresponding site on different receptors, or different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

Multivalent ligands may be prepared by either expressing amino acid 15 sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

Various combinations of amino acid sequences may be combined to 20 produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences that bind to the same site to form a multivalent ligand may be useful to produce molecules that are capable of cross-linking together 25 multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 7).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful 30 to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition, combining amino acid sequences that bind to different sites with different

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affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

In one embodiment, hybrids of at least two peptides (e.g., dimer 5 peptides) may be produced as recombinant fusion polypeptides, which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other sequences unrelated to ligand binding are 10 removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities 15 of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

In one embodiment shown below (Figure 28), MBP-FLAG®-PEPTIDE-(GGS)_n (SEQ ID NO: 1777)-PEPTIDE-E-TAG, a fusion construct producing a peptide dimer comprises a maltose binding protein amino acid 20 sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a FLAG® sequence to provide a cleavage site to remove the initial sequence. The dimer then follows which includes the intervening linker and a tag sequence 25 may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence. As non-limiting examples, n can be 1, 2, 3, or 4 to yield a linker sequence of 3, 6, 9, and 12 amino acids, respectively.

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In addition to producing the dimer peptides by recombinant protein expression, dimer peptides may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

- 5 Other methods of constructing dimer peptides include introducing a linker molecule that activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include, but are not limited to, diaminopropionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula O=CH-(CH₂)_n-CH=O, wherein n is at least 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Other preferred linkers are shown in Table 3. Linkers may be used, for example, to couple monomers at either the carboxyl terminal or the amino terminal ends to form dimer peptides. Also, the chemistry can be inverted, i.e., the peptides to be coupled can be equipped with aldehyde functions, either by oxidation with sodium periodate of an N-terminal serine, or by oxidation of any other vicinal hydroxy- or amino-groups, and the linker can comprise two oxyamino functions (e.g., at end of a polyethylene glycol linker) or amino groups which are coupled by reductive amination.
- 10 15 20 25 30
- In specific embodiments, Site 1-Site 2 and Site 2-Site 1 orientations are possible. In addition, N-terminal to N-terminal (N-N); C-terminal to C-terminal (C-C); N-terminal to C-terminal (N-C); and C-terminal to N-terminal (C-N) linkages are possible. Accordingly, peptides may be oriented Site 1 to Site 2, or Site 2 to Site 1, and may be linked N-terminus to N-terminus, C-terminus to C-terminus, N-terminus to C-terminus, or C-terminus to N-terminus. In certain cases, a specific orientation may be preferable to others, for example, for maximal agonist or antagonist activity.

- In an unexpected and surprising result, the orientation and linkage of the monomer subunits has been found to dramatically alter dimer activity (see Examples, below). In particular, certain Site 1/Site 2 heterodimer sequences show agonist or antagonist activity at IR, depending on

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orientation and linkage of the constituent monomer subunits. For example, a Site 1-Site 2 orientation (C-N linkage), e.g., the S453 heterodimer, shows antagonist activity at IR (Figure 18A; Table 7). In contrast, a Site 2-Site 1 orientation (C-N linkage), e.g., the S455 heterodimer, shows potent agonist 5 activity at IR (Figure 18D; Table 7). Similarly, Site 1-Site 2 (C-N linkage) heterodimers, e.g., S425 and S459, show antagonist activity at IR (Table 7), while Site 1-Site 2 (C-C or N-N linkage) heterodimers, e.g., S432-S438, S454, and S456, show agonist activity (Table 7).

Whether produced by recombinant gene expression or by 10 conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids 15 corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for 20 producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

2. Characterization Of Specific Dimers

Specific dimers which are comprised of monomer subunits that both 25 bind with high affinity to the same site on IR (i.e., homodimers), or monomer subunits that bind to different sites on IR (i.e., heterodimers) are disclosed herein.

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

F. Peptide Synthesis

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention. The present invention encompasses 5 the specific amino acid sequences shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*, without additions (e.g., linker or spacer sequences) deletions, alterations, or modification. The present invention further encompasses variants that include additional sequences, altered sequences, and functional fragments thereof. In a preferred embodiment, the amino acid 10 sequence variant or fragment shares at least one function characteristic (e.g., binding, agonist, or antagonist activity) of the reference sequence. Variant peptides include, for example, genetically engineered mutants, and may differ from the amino acid sequences shown in the figures and tables of the application by the addition, deletion, or substitution of one or more 15 amino acid residues. Alterations may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In addition, variants may comprise synthetic 20 or non-naturally occurring amino acids in accordance with this invention.

Variant amino acid sequences can have conservative changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More infrequently, a variant peptide can have non-conservative changes, e.g., substitution of a 25 glycine with a tryptophan. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing binding or biological activity can be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR, Inc., Madison, WI). Guidance is also provided by the data disclosed herein. In particular, 30 Figures 1-4, 8, 9, 43, 44, and Table 7, *inter alia*, teach which amino acid residues can be deleted, added, substituted, or modified, while maintaining

the IR- or IGF-1R-related function(s) (e.g., binding, agonist, or antagonist activity) of the amino acid sequences.

- For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), leucine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R).
- Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T). As non-limiting examples, the amino acids within each of these defined groups may be substituted for each other in the formulas described above, as conservative substitutions, subject to the specific preferences stated herein.
- Substantial changes in function can be made by selecting substitutions that are less conservative than those shown in the defined groups, above. For example, non-conservative substitutions can be made which more significantly affect the structure of the peptide in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which generally are expected to produce the

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greatest changes in the peptide's properties are those where 1) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; 2) a cysteine or proline is substituted for (or by) any other residue; 3) a residue 5 having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or 4) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

Amino acid preferences have been identified for certain peptides and 10 peptide groups of the present invention. For example, amino acid preferences for the RP9, D8, and Group 6 (Formula 10) peptides are shown in Tables 17-19, below.

Variants also include amino acid sequences in which one or more residues are modified (i.e., by phosphorylation, sulfation, acylation, 15 PEGylation, etc.), and mutants comprising one or more modified residues. Amino acid sequences may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotope, fluorescent, and enzyme labels. Fluorescent labels include, for example, Cy3, Cy5, Alexa, BODIPY, fluorescein (e.g., FluorX, 20 DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Preferred isotope labels include ³ H, ¹⁴ C, ³² P, ³⁵ S, ³⁶ Cl, ⁵¹ Cr, ⁵⁷ Co, ⁵⁸ Co, ⁵⁹ Fe, ⁹⁰ Y, ¹²⁵ I, ¹³¹ I, and ¹⁸⁶ Re. Preferred enzyme labels include peroxidase, β -glucuronidase, β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase, and alkaline 25 phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric 30 techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSATM), are known in the art, and are commercially available

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(see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, CA; NEN® Life Science Products, Inc., Boston, MA).

1. Recombinant Synthesis of Peptides

To obtain recombinant peptides, DNA sequences encoding these
5 peptides may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see Sambrook et al., 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

A large number of vectors, including bacterial, yeast, and mammalian
10 vectors, have been described for replication and/or expression in various host cells or cell-free systems, and may be used for gene therapy as well as for simple cloning or protein expression. In one aspect of the present invention, an expression vector comprises a nucleic acid encoding a IR or IGF-1R agonist or antagonist peptide, as described herein, operably linked
15 to at least one regulatory sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D.V. Goeddel (1990) *Methods Enzymol.* 185:3-7). Enhancer and other
20 expression control sequences are described in *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1983). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of peptide desired to be expressed.

25 Several regulatory elements (e.g., promoters) have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Such regulatory regions, methods of isolation, manner of manipulation, etc. are known in the art. Non-limiting examples of bacterial promoters include the β-lactamase (penicillinase) promoter; lactose
30 promoter; tryptophan (trp) promoter; araBAD (arabinose) operon promoter;

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lambda-derived P₁ promoter and N gene ribosome binding site; and the hybrid tac promoter derived from sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include the 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactoepimerase promoter, and alcohol dehydrogenase (ADH1) promoter. Suitable promoters for mammalian cells include, without limitation, viral promoters, such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Preferred replication and inheritance systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, CEN ARS, 2μm ARS and the like. While expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

To obtain expression in eukaryotic cells, terminator sequences, polyadenylation sequences, and enhancer sequences that modulate gene expression may be required. Sequences that cause amplification of the gene may also be desirable. Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or preprotein or proprotein sequences, may also be included. These sequences are well described in the art. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism or expression system. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using well-established techniques.

Codon usage data can be obtained from publicly-available sources, for example, the Codon Usage Database at <http://www.kazusa.or.jp/codon/>. In addition, computer programs that translate amino acid sequence information into nucleotide sequence information in accordance with codon preferences (i.e., backtranslation programs) are widely available. See, for example, Backtranslate program from Genetics Computer Group (GCG),

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Accelrys, Inc., Madison, WI; and Backtranslation Applet from Entelechon GmbH, Regensburg, Germany. Thus, using the peptide sequences disclosed herein, one of ordinary skill in the art can design nucleic acids to yield optimal expression levels in the translation system or host cell of
5 choice.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells that express the inserts. Typical selection genes
10 encode proteins that 1) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; 2) complement auxotrophic deficiencies, or 3) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. Markers may be an inducible or non-inducible gene and will generally allow
15 for positive selection. Non-limiting examples of markers include the ampicillin resistance marker (i.e., beta-lactamase), tetracycline resistance marker, neomycin/kanamycin resistance marker (i.e., neomycin phosphotransferase), dihydrofolate reductase, glutamine synthetase, and the like. The choice of the proper selectable marker will depend on the host
20 cell, and appropriate markers for different hosts as understood by those of skill in the art.

Suitable expression vectors for use with the present invention include, but are not limited to, pUC, pBluescript (Stratagene), pET (Novagen, Inc., Madison, WI), and pREP (Invitrogen) plasmids. Vectors can
25 contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to
30 transcriptional regulatory elements (e.g., promoters, enhancers, and/or

insulators) and/or to other amino acid encoding sequences can be carried out using established methods.

Suitable cell-free expression systems for use with the present invention include, without limitation, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). These systems allow the expression of recombinant peptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing protein-coding regions and appropriate promoter elements.

Non-limiting examples of suitable host cells include bacteria, archaea, insect, fungi (e.g., yeast), plant, and animal cells (e.g., mammalian, especially human). Of particular interest are *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines.

Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (Eds), 1979, *Cell Culture. Methods in Enzymology*, volume 58, Academic Press, Inc., Harcourt Brace Jovanovich, NY). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be used, e.g., to provide higher expression, or other features.

Host cells can be transformed, transfected, or infected as appropriate by any suitable method including electroporation, calcium chloride-, lithium chloride-, lithium acetate/polyethylene glycol-, calcium phosphate-, DEAE-dextran-, liposome-mediated DNA uptake, spheroplasting, injection, microinjection, microprojectile bombardment, phage infection, viral infection, or other established methods. Alternatively, vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo *et al.*, 1988, *FEBS Letts.* 241:119). The cells into which have been

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introduced nucleic acids described above are meant to also include the progeny of such cells.

Nucleic acids encoding the peptides of the invention may be isolated directly from recombinant phage libraries (e.g., RAPIDLIB® or GRABLIB® libraries) described herein. Alternatively, the polymerase chain reaction (PCR) method can be used to produce nucleic acids of the invention, using the recombinant phage libraries as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

Nucleic acids encoding the peptides of the present invention can also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage *et al.*, 1981, *Tetra. Letts.* 22:1859-1862, or the triester method according to Matteucci *et al.*, 1981, *J. Am. Chem. Soc.*, 103:3185, and can be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

The nucleic acids encoding the peptides of the invention can be produced in large quantities by replication in a suitable host cell. Natural or synthetic nucleic acid fragments, comprising at least ten contiguous bases coding for a desired amino acid sequence can be incorporated into recombinant nucleic acid constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the nucleic acid constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cells, cell lines, tissues, or organisms. The purification of nucleic acids produced by the methods of the present

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invention is described, for example, in Sambrook et al., 1989; F.M. Ausubel et al., 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, NY.

These nucleic acids can encode variant or truncated forms of the peptides as well as the reference peptides shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*. Large quantities of the nucleic acids and peptides of the present invention may be prepared by expressing the nucleic acids or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. For example, insect cell systems (i.e., lepidopteran host cells and baculovirus expression vectors) are particularly suited for large-scale protein production.

Host cells carrying an expression vector (i.e., transformants or clones) are selected using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

For some purposes, it is preferable to produce the peptide in a recombinant system in which the peptide contains an additional sequence (e.g., epitope or protein) tag that facilitates purification. Non-limiting examples of epitope tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS)(SEQ ID NO: 1778), GLU-GLU, and DYKDDDDK (SEQ ID NO:1779) or DYKD (SEQ ID NO:1545; FLAG®) epitope tags. Non-limiting examples of protein tags include glutathione-S-transferase (GST), green fluorescent protein (GFP), and maltose binding protein (MBP). In one

approach, the coding sequence of a peptide can be cloned into a vector that creates a fusion with a sequence tag of interest. Suitable vectors include, without limitation, pRSET (Invitrogen Corp., San Diego, CA), pGEX (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), pEGFP (CLONTECH Laboratories, Inc., Palo Alto, CA), and pMALTM (New England BioLabs, Inc., Beverly, MA) plasmids. Following expression, the epitope or protein tagged peptide can be purified from a crude lysate of the translation system or host cell by chromatography on an appropriate solid-phase matrix. In some cases, it may be preferable to remove the epitope or protein tag (i.e., via protease cleavage) following purification.

Methods for directly purifying peptides from sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

Antibody-based methods may also be used to purify peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

2. Chemical Synthesis Of Peptides

Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The peptides are preferably prepared by solid-

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phase peptide synthesis; for example, as described by Merrifield (1965; 1997).

According to methods known in the art, peptides can be chemically synthesized by commercially available automated procedures, including, 5 without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation, classical solution synthesis. In addition, recombinant and synthetic methods of peptide production can be combined to produce semi-synthetic peptides. The peptides of the invention are preferably prepared by solid phase peptide synthesis as described by 10 Merrifield, 1963, *J. Am. Chem. Soc.* 85:2149; 1997. In one embodiment, synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the peptides. The alpha-amino protecting 15 group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise peptide synthesis. Included are acyl type protecting 20 groups, e.g., formyl, trifluoroacetyl, acetyl, aromatic urethane type protecting groups, e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc), aliphatic urethane protecting groups, e.g., t-butyloxycarbonyl (Boc), isopropylloxycarbonyl, cyclohexyloxycarbonyl, and alkyl type protecting groups, e.g., benzyl, triphenylmethyl. The 25 preferred protecting group is Boc. The side-chain protecting groups for Tyr include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl, and cyclohexyl. The preferred side-chain 30 protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-

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dichlorobenzyl, and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantlyloxycarbonyl, and Boc. The preferred protecting group for Arg is Tos. The side-chain amino group of Lys can be protected with Cbz, 2-Cl-Cbz, Tos, or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished peptide.

Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting peptide will have a free carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting peptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart *et al.*, 1984, *Solid Phase Peptide Synthesis* (2nd Edition), Pierce Chemical Co., Rockford, IL.

The C-terminal amino acid, protected at the side chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropyl-carbodiimide and carbonyldiimidazole. Following the attachment to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0 and 25°C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

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Various activating agents can be used for the coupling reactions including DCC,N,N'-diisopropyl-carbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexa-fluorophosphate (BOP) and DCC-hydroxybenzotriazole (HO_Bt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser *et al.*, 1970, *Anal. Biochem.* 34:595. In cases where incomplete coupling is found, the coupling reaction is repeated. The coupling reactions can be performed automatically with commercially available instruments.

After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent such as liquid HF for 1-2 hours at 0°C, which cleaves the peptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage from alkylating the amino acid residues present in the peptide. The peptide-resin can be deprotected with TFA/dithioethane prior to cleavage if desired.

Side-chain to side-chain cyclization on the solid support requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected peptide-resin are selectively removed with piperidine in DMF. Cyclization is achieved on the solid support using various activating agents including DCC, DCC/HO_Bt, or BOP. The HF reaction is carried out on the cyclized peptide-resin as described above.

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3. Peptide Libraries

Peptide libraries produced and screened according to the present invention are useful in providing new ligands for IR and IGF-1R. Peptide libraries can be designed and panned according to methods described in 5 detail herein, and methods generally available to those in the art (see, e.g., U.S. Patent No. 5,723,286 issued March 3, 1998 to Dower *et al.*). In one aspect, commercially available phage display libraries can be used (e.g., RAPIDLIB® or GRABLIB®, DGI BioTechnologies, Inc., Edison, NJ; Ph.D. C7C Disulfide Constrained Peptide Library, New England Biolabs). In 10 another aspect, an oligonucleotide library can be prepared according to methods known in the art, and inserted into an appropriate vector for peptide expression. For example, vectors encoding a bacteriophage structural protein, preferably an accessible phage protein, such as a bacteriophage coat protein, can be used. Although one skilled in the art will appreciate that 15 a variety of bacteriophage may be employed in the present invention, in preferred embodiments the vector is, or is derived from, a filamentous bacteriophage, such as, for example, f1, fd, Pf1, M13, etc. In particular, the fd-tet vector has been extensively described in the literature (see, e.g., Zacher *et al.*, 1980, *Gene* 9:127-140; Smith *et al.*, 1985, *Science* 228:1315- 20 1317; Parmley and Smith, 1988, *Gene* 73:305-318).

The phage vector is chosen to contain or is constructed to contain a cloning site located in the 5' region of the gene encoding the bacteriophage structural protein, so that the peptide is accessible to receptors in an affinity enrichment procedure as described hereinbelow. The structural phage 25 protein is preferably a coat protein. An example of an appropriate coat protein is pIII. A suitable vector may allow oriented cloning of the oligonucleotide sequences that encode the peptide so that the peptide is expressed at or within a distance of about 100 amino acid residues of the N-terminus of the mature coat protein. The coat protein is typically expressed 30 as a preprotein, having a leader sequence.

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Thus, desirably the oligonucleotide library is inserted so that the N-terminus of the processed bacteriophage outer protein is the first residue of the peptide, i.e., between the 3'-terminus of the sequence encoding the leader protein and the 5'-terminus of the sequence encoding the mature protein or a portion of the 5' terminus. The library is constructed by cloning an oligonucleotide which contains the variable region of library members (and any spacers, as discussed below) into the selected cloning site. Using known recombinant DNA techniques (see generally, Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), an oligonucleotide may be constructed which, *inter alia*; 1) removes unwanted restriction sites and adds desired ones; 2) reconstructs the correct portions of any sequences which have been removed (such as a correct signal peptidase site, for example); 3) inserts the spacer residues, if any; and/or 4) corrects the translation frame (if necessary) to produce active, infective phage.

The central portion of the oligonucleotide will generally contain one or more IR and/or IGF-1R binding sequences and, optionally, spacer sequences. The sequences are ultimately expressed as peptides (with or without spacers) fused to or in the N-terminus of the mature coat protein on the outer, accessible surface of the assembled bacteriophage particles. The size of the library will vary according to the number of variable codons, and hence the size of the peptides, which are desired. Generally the library will be at least about 10^6 members, usually at least 10^7 , and typically 10^8 or more members. To generate the collection of oligonucleotides which forms a series of codons encoding a random collection of amino acids and which is ultimately cloned into the vector, a codon motif is used, such as (NNK)_x, where N may be A, C, G, or T (nominally equimolar), K is G or T (nominally equimolar), and x is typically up to about 5, 6, 7, 8, or more, thereby producing libraries of penta-, hexa-, hepta-, and octa-peptides or larger.

30 The third position may also be G or C, designated "S". Thus, NNK or NNS

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1) code for all the amino acids; 2) code for only one stop codon; and 3) reduce the range of codon bias from 6:1 to 3:1.

It should be understood that, with longer peptides, the size of the library that is generated may become a constraint in the cloning process.

- 5 The expression of peptides from randomly generated mixtures of oligonucleotides in appropriate recombinant vectors is known in the art (see, e.g., Oliphant *et al.*, Gene 44:177-183). For example, the codon motif (NNK)₆ produces 32 codons, one for each of 12 amino acids, two for each of five amino acids, three for each of three amino acids and one (amber) stop 10 codon. Although this motif produces a codon distribution as equitable as available with standard methods of oligonucleotide synthesis, it results in a bias against peptides containing one-codon residues. In particular, a complete collection of hexacodons contains one sequence encoding each peptide made up of only one-codon amino acids, but contains 729 (3^6) 15 sequences encoding each peptide with only three-codon amino acids.

An alternative approach to minimize the bias against one-codon residues involves the synthesis of 20 activated trinucleotides, each representing the codon for one of the 20 genetically encoded amino acids. These are synthesized by conventional means, removed from the support 20 while maintaining the base and 5-OH-protecting groups, and activated by the addition of 3'O-phosphoramidite (and phosphate protection with β -cyanoethyl groups) by the method used for the activation of mononucleosides (see, generally, McBride and Caruthers, 1983, *Tetrahedron Letters* 22:245). Degenerate oligocodons are prepared using 25 these trimers as building blocks. The trimers are mixed at the desired molar ratios and installed in the synthesizer. The ratios will usually be approximately equimolar, but may be a controlled unequal ratio to obtain the over- to under-representation of certain amino acids coded for by the degenerate oligonucleotide collection. The condensation of the trimers to 30 form the oligocodons is done essentially as described for conventional synthesis employing activated mononucleosides as building blocks (see,

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e.g., Atkinson and Smith, 1984, *Oligonucleotide Synthesis*, M.J. Gait, Ed., p. 35-82). This procedure generates a population of oligonucleotides for cloning that is capable of encoding an equal distribution (or a controlled unequal distribution) of the possible peptide sequences. Advantageously, 5 this approach may be employed in generating longer peptide sequences, since the range of bias produced by the (NNK)₆ motif increases by three-fold with each additional amino acid residue.

When the codon motif is (NNK)_x, as defined above, and when x equals 8, there are 2.6×10^{10} possible octa-peptides. A library containing 10 most of the octa-peptides may be difficult to produce. Thus, a sampling of the octa-peptides may be accomplished by constructing a subset library using up to about 10% of the possible sequences, which subset of recombinant bacteriophage particles is then screened. If desired, to extend 15 the diversity of a subset library, the recovered phage subset may be subjected to mutagenesis and then subjected to subsequent rounds of screening. This mutagenesis step may be accomplished in two general ways: the variable region of the recovered phage may be mutagenized, or additional variable amino acids may be added to the regions adjoining the initial variable sequences.

20 To diversify around active peptides (i.e., binders) found in early rounds of panning, the positive phage can be sequenced to determine the identity of the active peptides. Oligonucleotides can then be synthesized based on these peptide sequences. The syntheses are done with a low level of all bases incorporated at each step to produce slight variations of 25 the primary oligonucleotide sequences. This mixture of (slightly) degenerate oligonucleotides can then be cloned into the affinity phage by methods known to those in the art. This method produces systematic, controlled variations of the starting peptide sequences as part of a secondary library. It requires, however, that individual positive phage be sequenced before 30 mutagenesis, and thus is useful for expanding the diversity of small numbers of recovered phage.

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An alternate approach to diversify the selected phage allows the mutagenesis of a pool, or subset, of recovered phage. In accordance with this approach, phage recovered from panning are pooled and single stranded DNA is isolated. The DNA is mutagenized by treatment with, e.g.,

5 nitrous acid, formic acid, or hydrazine. These treatments produce a variety of damage to the DNA. The damaged DNA is then copied with reverse transcriptase, which misincorporates bases when it encounters a site of damage. The segment containing the sequence encoding the receptor-binding peptide is then isolated by cutting with restriction nuclease(s)

10 specific for sites flanking the peptide coding sequence. This mutagenized segment is then recloned into undamaged vector DNA, the DNA is transformed into cells, and a secondary library according to known methods. General mutagenesis methods are known in the art (see Myers *et al.*, 1985, *Nucl. Acids Res.* 13:3131-3145; Myers *et al.*, 1985, *Science* 229:242-246;

15 Myers, 1989, *Current Protocols in Molecular Biology Vol. I*, 8.3.1-8.3.6, F. Ausubel *et al.*, eds, J. Wiley and Sons, New York).

In another general approach, the addition of amino acids to a peptide or peptides found to be active, can be carried out using various methods. In one, the sequences of peptides selected in early panning are determined

20 individually and new oligonucleotides, incorporating the determined sequence and an adjoining degenerate sequence, are synthesized. These are then cloned to produce a secondary library. Alternatively, methods can be used to add a second IR or IGF-1R binding sequence to a pool of peptide-bearing phage. In accordance with one method, a restriction site is

25 installed next to the first IR or IGF-1R binding sequence. Preferably, the enzyme should cut outside of its recognition sequence. The recognition site may be placed several bases from the first binding sequence. To insert a second IR or IGF-1R binding sequence, the pool of phage DNA is digested and blunt-ended by filling in the overhang with Klenow fragment. Double-

30 stranded, blunt-ended, degenerately synthesized oligonucleotides are then ligated into this site to produce a second binding sequence juxtaposed to the

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first binding sequence. This secondary library is then amplified and screened as before.

While in some instances it may be appropriate to synthesize longer peptides to bind certain receptors, in other cases it may be desirable to 5 provide peptides having two or more IR or IGF-1R binding sequences separated by spacer (e.g., linker) residues. For example, the binding sequences may be separated by spacers that allow the regions of the peptides to be presented to the receptor in different ways. The distance between binding regions may be as little as 1 residue, or at least 2-20 10 residues, or up to at least 100 residues. Preferred spacers are 3, 6, 9, 12, 15, or 18 residues in length. For probing large binding sites or tandem 15 binding sites (e.g., Site 1 and Site 2 of IR), the binding regions may be separated by a spacer of residues of up to 20 to 30 amino acids. The number of spacer residues when present will typically be at least 2 residues, and often will be less than 20 residues.

The oligonucleotide library may have binding sequences which are separated by spacers (e.g., linkers), and thus may be represented by the formula: $(NNK)^y - (abc)_n - (NNK)_z$ where N and K are as defined previously (note that S as defined previously may be substituted for K), and $y+z$ is 20 equal to about 5, 6, 7, 8, or more, a, b and c represent the same or different nucleotides comprising a codon encoding spacer amino acids, n is up to about 3, 6, 9, or 12 amino acids, or more. The spacer residues may be somewhat flexible, comprising oligo-glycine, or oligo-glycine-glycine-serine, for example, to provide the diversity domains of the library with the ability to 25 interact with sites in a large binding site relatively unconstrained by attachment to the phage protein. Rigid spacers, such as, e.g., oligo-proline, may also be inserted separately or in combination with other spacers, including glycine spacers. It may be desired to have the IR or IGF-1R binding sequences close to one another and use a spacer to orient the 30 binding sequences with respect to each other, such as by employing a turn between the two sequences, as might be provided by a spacer of the

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sequence glycine-proline-glycine, for example. To add stability to such a turn, it may be desirable or necessary to add cysteine residues at either or both ends of each variable region. The cysteine residues would then form disulfide bridges to hold the variable regions together in a loop, and in this
5 fashion may also serve to mimic a cyclic peptide. Of course, those skilled in the art will appreciate that various other types of covalent linkages for cyclization may also be used.

Spacer residues as described above may also be situated on either or both ends of the IR or IGF-1R binding sequences. For instance, a cyclic
10 peptide may be designed without an intervening spacer, by having a cysteine residue on both ends of the peptide. As described above, flexible spacers, e.g., oligo-glycine, may facilitate interaction of the peptide with the selected receptors. Alternatively, rigid spacers may allow the peptide to be presented as if on the end of a rigid arm, where the number of residues,
15 e.g., proline residues, determines not only the length of the arm but also the direction for the arm in which the peptide is oriented. Hydrophilic spacers, made up of charged and/or uncharged hydrophilic amino acids, (e.g., Thr, His, Asn, Gln, Arg, Glu, Asp, Met, Lys, etc.), or hydrophobic spacers of hydrophobic amino acids (e.g., Phe, Leu, Ile, Gly, Val, Ala, etc.) may be
20 used to present the peptides to receptor binding sites with a variety of local environments.

Notably, some peptides, because of their size and/or sequence, may cause severe defects in the infectivity of their carrier phage. This causes a loss of phage from the population during reinfection and amplification
25 following each cycle of panning. To minimize problems associated with defective infectivity, DNA prepared from the eluted phage can be transformed into appropriate host cells, such as, e.g., *E. coli*, preferably by electroporation (see, e.g., Dower *et al.*, *Nucl. Acids Res.* **16**:6127-6145), or well known chemical means. The cells are cultivated for a period of time
30 sufficient for marker expression, and selection is applied as typically done for DNA transformation. The colonies are amplified, and phage harvested

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for affinity enrichment in accordance with established methods. Phage identified in the affinity enrichment may be re-amplified by infection into the host cells. The successful transformants are selected by growth in an appropriate antibiotic(s), e.g., tetracycline or ampicillin. This may be done
5 on solid or in liquid growth medium.

For growth on solid medium, the cells are grown at a high density (about 10^8 to 10^9 transformants per m²) on a large surface of, for example, L-agar containing the selective antibiotic to form essentially a confluent lawn. The cells and extruded phage are scraped from the surface and
10 phage are prepared for the first round of panning (see, e.g., Parmley and Smith, 1988, *Gene* 73:305-318). For growth in liquid culture, cells may be grown in L-broth and antibiotic through about 10 or more doublings. The
15 phage are harvested by standard procedures (see Sambrook *et al.*, 1989, *Molecular Cloning*, 2nd ed.). Growth in liquid culture may be more convenient because of the size of the libraries, while growth on solid media likely provides less chance of bias during the amplification process.

For affinity enrichment of desired clones, generally about 10^3 to 10^4 library equivalents (a library equivalent is one of each recombinant; 10^4 equivalents of a library of 10^9 members is $10^9 \times 10^4 = 10^{13}$ phage), but
20 typically at least 10^2 library equivalents, up to about 10^5 to 10^6 , are incubated with a receptor (or portion thereof) to which the desired peptide is sought. The receptor is in one of several forms appropriate for affinity enrichment schemes. In one example the receptor is immobilized on a surface or particle, and the library of phage bearing peptides is then panned
25 on the immobilized receptor generally according to procedures known in the art. In an alternate scheme, a receptor is attached to a recognizable ligand (which may be attached via a tether). A specific example of such a ligand is biotin. The receptor, so modified, is incubated with the library of phage and binding occurs with both reactants in solution. The resulting complexes are
30 then bound to streptavidin (or avidin) through the biotin moiety. The streptavidin may be immobilized on a surface such as a plastic plate or on

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particles, in which case the complexes (phage/peptide/receptor/biotin/streptavidin) are physically retained; or the streptavidin may be labeled, with a fluorophor, for example, to tag the active phage/peptide for detection and/or isolation by sorting procedures, e.g., on a 5 fluorescence-activated cell sorter.

Phage that associate with IR or IGF-1R via non-specific interactions are removed by washing. The degree and stringency of washing required will be determined for each receptor/peptide of interest. A certain degree of control can be exerted over the binding characteristics of the peptides 10 recovered by adjusting the conditions of the binding incubation and the subsequent washing. The temperature, pH, ionic strength, divalent cation concentration, and the volume and duration of the washing will select for peptides within particular ranges of affinity for the receptor. Selection based on slow dissociation rate, which is usually predictive of high affinity, is the 15 most practical route. This may be done either by continued incubation in the presence of a saturating amount of free ligand, or by increasing the volume, number, and length of the washes. In each case, the rebinding of dissociated peptide-phage is prevented, and with increasing time, peptide-phage of higher and higher affinity are recovered. Additional modifications 20 of the binding and washing procedures may be applied to find peptides that bind receptors under special conditions. Once a peptide sequence that imparts some affinity and specificity for the receptor molecule is known, the diversity around this binding motif may be embellished. For instance, variable peptide regions may be placed on one or both ends of the identified 25 sequence. The known sequence may be identified from the literature, or may be derived from early rounds of panning in the context of the present invention.

G. Screening Assays

In another embodiment of this invention, screening assays to identify 30 pharmacologically active ligands at IR and/or IGF-1R are provided. Ligands

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may encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Such ligands can comprise functional groups necessary for structural interaction with

5 proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. Ligands often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Ligands can

10 also comprise biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs, or combinations thereof.

Ligands may include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide

15 libraries (see, e.g., Lam *et al.*, 1991, *Nature* 354:82-84; Houghten *et al.*, 1991, *Nature* 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang *et al.*, 1993, *Cell* 72:767-778);

20 3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies); and 4) small organic and inorganic molecules.

Ligands can be obtained from a wide variety of sources including

25 libraries of synthetic or natural compounds. Synthetic compound libraries are commercially available from, for example, Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, WI).

30 Natural compound libraries comprising bacterial, fungal, plant or animal extracts are available from, for example, Pan Laboratories (Bothell, WA). In

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addition, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

Alternatively, libraries of natural compounds in the form of bacterial,
5 fungal, plant and animal extracts can be readily produced. Methods for the
synthesis of molecular libraries are readily available (see, e.g., DeWitt *et al.*,
1993, *Proc. Natl. Acad. Sci. USA* **90**:6909; Erb *et al.*, 1994, *Proc. Natl. Acad.
Sci. USA* **91**:11422; Zuckermann *et al.*, 1994, *J. Med. Chem.* **37**:2678; Cho
et al., 1993, *Science* **261**:1303; Carell *et al.*, 1994, *Angew. Chem. Int. Ed.
Engl.* **33**:2059; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* **33**:2061; and
in Gallop *et al.*, 1994, *J. Med. Chem.* **37**:1233). In addition, natural or
synthetic compound libraries and compounds can be readily modified
through conventional chemical, physical and biochemical means (see, e.g.,
Blondelle *et al.*, 1996, *Trends in Biotech.* **14**:60), and may be used to
15 produce combinatorial libraries. In another approach, previously identified
pharmacological agents can be subjected to directed or random chemical
modifications, such as acylation, alkylation, esterification, amidification, and
the analogs can be screened for IR-modulating activity.

Numerous methods for producing combinatorial libraries are known in
20 the art, including those involving biological libraries; spatially addressable
parallel solid phase or solution phase libraries; synthetic library methods
requiring deconvolution; the 'one-bead one-compound' library method; and
synthetic library methods using affinity chromatography selection. The
biological library approach is limited to polypeptide or peptide libraries, while
25 the other four approaches are applicable to polypeptide, peptide, non-
peptide oligomer, or small molecule libraries of compounds (K. S. Lam,
1997, *Anticancer Drug Des.* **12**:145).

Libraries may be screened in solution by methods generally known in
the art for determining whether ligands competitively bind at a common
30 binding site. Such methods may include screening libraries in solution
(e.g., Houghten, 1992, *Biotechniques* **13**:412-421), or on beads (Lam, 1991,

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Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria or spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869), or on phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 5 1990, Proc. Natl. Acad. Sci. USA 97:6378-6382; Felici, 1991, J. Mol. Biol. 222:301-310; Ladner, *supra*).

Where the screening assay is a binding assay, IR, or one of the IR-binding peptides disclosed herein, may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include 10 radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes, specific binding molecules, particles, e.g., magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin, etc. For the specific binding members, the complementary member would normally be labeled with a molecule that 15 provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc., which are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the 20 efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The components are added in any order that produces the requisite binding. Incubations are performed at any temperature that facilitates optimal activity, typically between 4° and 40°C. Incubation periods are selected for optimum activity, but may also be 25 optimized to facilitate rapid high-throughput screening. Normally, between 0.1 and 1 hr will be sufficient. In general, a plurality of assay mixtures is run in parallel with different test agent concentrations to obtain a differential response to these concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of 30 detection.

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The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557, which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides that bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region that has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display (see WO 96/04557 and Kay *et al.* 1996, *Mol. Divers.* 1(2):139-40, both of which are incorporated herein by reference). The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993, *J. Immunol. Methods* 163:209-216). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% bovine serum albumin (BSA) and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab.

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Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu³⁺ which rapidly forms a highly fluorescent and stable complex with components of the enhancement 5 solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.).

Phage display libraries can also be screened for ligands that bind to IR or IGF-1R, as described above. Details of the construction and analyses 10 of these libraries, as well as the basic procedures for biopanning and selection of binders, have been published (see, e.g., WO 96/04557; Mandecki *et al.*, 1997, *Display Technologies – Novel Targets and Strategies*, P. Gutfry (ed), International Business Communications, Inc. Southborogh, MA, pp. 231-254; Raveri *et al.*, 1998, *Oncogene* 16:1993-15 1999; Scott and Smith, 1990, *Science* 249:386-390); Grihalde *et al.*, 1995, *Gene* 166:187-195; Chen *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:1997-2001; Kay *et al.*, 1993, *Gene* 128:59-65; Carcamo *et al.*, 1998, *Proc. Natl. Acad. Sci. USA* 95:11146-11151; Hoogenboom, 1997, *Trends Biotechnol.* 15:62-70; Rader and Barbas, 1997, *Curr. Opin. Biotechnol.* 8:503-508; all of 20 which are incorporated herein by reference).

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for 25 a particular method of administration, e.g., peptides are generally unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis, and testing are generally used to avoid large-scale screening of molecules for a target property.

30 There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts

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of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g., by substituting each residue in turn). These parts or residues constituting the 5 active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g., stereochemistry, bonding, size, and/or charge), using data from a range of sources (e.g., spectroscopic techniques, X-ray diffraction data, and NMR). Computational analysis, 10 similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful 15 where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected, and chemical groups that mimic the pharmacophore can be grafted onto the template. The template molecule and the chemical groups grafted on to it can conveniently be 20 selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried 25 out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences that function as either agonists or antagonists at IR and/or IGF-1R. Additional sequences may be obtained in accordance with the procedures described herein.

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H. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, 5 for example, competition screening assays, as research tools for further analysis of IR and IGF-1R, and as potential therapeutics in pharmaceutical compositions. In one embodiment, one or more of the disclosed peptides can be provided as components in a kit for identifying other ligands (e.g., small, organic molecules) that bind to IR or IGF-1R. Such kits may also 10 comprise IR or IGF-1R, or functional fragments thereof. The peptide and receptor components of the kit may be labeled (e.g., by radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes or other labels), or may be unlabeled and labeling reagents may be provided. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. 15 Instructions for use can also be provided.

In another embodiment, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which are derived from the peptide sequences, and include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify 20 sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R, as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000, in accordance with well-established techniques.

25 IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, non-limiting examples of amino acid sequences include D117/H2C: 30 FHENFYDWFVRQVSK (SEQ ID NO:1780); D117/H2C minus terminal lysine: FHENFYDWFVRQVS (SEQ ID NO:1557); D118:

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DYKDFYDAIQLVRSARAGGTRDKK (SEQ ID NO:1781); D118 minus FLAG® tag and terminal lysines: FYDAIQLVRSARAGGTRD (SEQ ID NO:1782); D119: KDRAFYNGLRDLVGAVYGAWDKK (SEQ ID NO:1733); D119 minus terminal lysines: KDRAFYNGLRDLVGAVYGAWD (residues 1-5 21 of SEQ ID NO: 1733); D116/JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541); D116/JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542); D113/H2: DYKDVTFTSAVFHENFYDWFWVRQVSKK (SEQ ID NO:1783); D113/H2 minus FLAG® tag and terminal lysines: VTFTSAVFHENFYDWFWVRQVS 10 (SEQ ID NO:1784); and S175: GRVDWLQRNANFYDWFWVAELG (SEQ ID NO:1560). Preferred peptide dimer sequences are represented by S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418 (see Table 7). Other preferred dimers sequences are represented by S455, S457, S458, S467, S468, S471, S499, S510, S518, 15 S519, and S520 sequences (see Table 7). Especially preferred is the S519 dimer sequence, which shows *in vitro* and *in vivo* activity comparable to insulin (see Figures 31A-C, 32A-B, and 33), S557 (see, e.g., Figure 55), and S597 (see, e.g., Figures 54-56).

IGF-1R antagonist amino acid sequences provided by this invention 20 are useful as treatments for cancers, including, but not limited to, breast, prostate, colorectal, and ovarian cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein 25 are also useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999, *Nat. Med.* 5:1390-1395).

IGF-1R agonist amino acid sequences provided by this invention are 30 useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups

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implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer et al., 1998, *Neurology* 51:S39-S43; Apfel, 1999, *Am. J. Med.* 107:34S-42S).

I. Modification of Peptides

5 The peptides of the invention may be subjected to one or more modifications known in the art, which may be useful for manipulating storage stability, pharmacokinetics, and/or any aspect of the bioactivity of the peptide, such as, e.g., potency, selectivity, and drug interaction. Chemical modification to which the peptides may be subjected includes, without limitation, the conjugation to a peptide of one or more of polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polypropylene glycol, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, colominic acids or other carbohydrate based polymers, polymers of amino acids, and biotin derivatives. PEG conjugation of proteins at Cys residues is disclosed, e.g., in Goodson, R. J. & Katre, N. V. (1990) *Bio/Technology* 8, 343 and Kogan, T. P. (1992) *Synthetic Comm.* 22, 2417.

20 Other useful modifications include, without limitation, acylation, using methods and compositions such as described in, e.g., U.S. Patent Serial No. 6,251, 856, and WO 00/55119.

J. Therapeutic Administration

25 The peptides of the present invention may be administered individually or in combination with other pharmacologically active agents. It will be understood that such combination therapy encompasses different therapeutic regimens, including, without limitation, administration of multiple agents together in a single dosage form or in distinct, individual dosage forms. If the agents are present in different dosage forms, administration

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may be simultaneous or near-simultaneous or may follow any predetermined regimen that encompasses administration of the different agents.

- For example, when used to treat diabetes or other diseases or syndromes associated with a decreased response or production of insulin, hyperlipidemia, obesity, appetite-related syndromes, and the like, the peptides of the invention may be advantageously administered in a combination treatment regimen with one or more agents, including, without limitation, insulin, insulin analogues, insulin derivatives, glucagon-like peptide-1 or-2 (GLP-1, GLP-2), derivatives or analogues of GLP-1 or GLP-2 (such as are disclosed, e.g., in WO 00/55119). It will be understood that an "analogue" of insulin, GLP-1, or GLP-2 as used herein refers to a peptide containing one or more amino acid substitutions relative to the native sequence of insulin, GLP-1, or GLP-2, as applicable; and "derivative" of insulin, GLP-1, or GLP-2 as used herein refers to a native or analogue insulin, GLP-1, or GLP-2 peptide that has undergone one or more additional chemical modifications of the amino acid sequence, in particular relative to the natural sequence. Insulin derivatives and analogues are disclosed, e.g., in U.S. Patent Serial No. 5,656,722, 5,750,497, 6,251,856, and 6,268,335.
- In some embodiments, the combination agent is one of Lys^{B29}(-myristoyl)des(B30) human insulin, Lys^{B29}(-tetradecanoyl)des(B30) human insulin and B²⁹-N -(N-lithocolyl- -glutamyl)-des(B30) human insulin. Also suitable for combination therapy are non-peptide antihyperglycemic agents, antihyperlipidemic agents, and the like such as those well-known in the art.
- In one embodiment, the invention encompasses methods of treating diabetes or related syndromes comprising administering a first amount of peptide S597 or peptide S557 and a second amount of a long-acting insulin analogue, such as, e.g., Lys^{B29}(-myristoyl)des(B30) human insulin, Lys^{B29}(-tetradecanoyl)des(B30) human insulin, or B²⁹-N -(N-lithocolyl- -glutamyl)-des(B30) human insulin, wherein the first and second amounts together are effective for treating the syndrome. As used herein, a long-

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acting insulin analogue is one that exhibits a protracted profile of action relative to native human insulin, as disclosed, e.g., in U.S. Patent Serial No. 6,451,970.

The peptides of the present invention may be administered
5 individually or in combination with other pharmacologically active agents. It will be understood that such combination therapy encompasses different therapeutic regimens, including, without limitation, administration of multiple agents together in a single dosage form or in distinct, individual dosage forms. If the agents are present in different dosage forms, administration
10 may be simultaneous or near-simultaneous or may follow any predetermined regimen that encompasses administration of the different agents.

For example, when used to treat diabetes or other diseases or syndromes associated with a decreased response or production of insulin,
15 hyperlipidemia, obesity, appetite-related syndromes, and the like, the peptides of the invention may be advantageously administered in a combination treatment regimen with one or more agents, including, without limitation, insulin, insulin analogues, insulin derivatives, glucagon-like peptide-1 or-2 (GLP-1, GLP-2), derivatives or analogues of GLP-1 or GLP-2
20 (such as are disclosed, e.g., in WO 00/55119). It will be understood that an "analogue" of insulin, GLP-1, or GLP-2 as used herein refers to a peptide containing one or more amino acid substitutions relative to the native sequence of insulin, GLP-1, or GLP-2, as applicable; and "derivative" of insulin, GLP-1, or GLP-2 as used herein refers to a native or analogue
25 insulin, GLP-1, or GLP-2 peptide that has undergone one or more additional chemical modifications of the amino acid sequence, in particular relative to the natural sequence. Insulin derivatives and analogues are disclosed, e.g., in U.S. Patent Serial No. 5,656,722, 5,750,497, 6,251,856, and 6,268,335.
In some embodiments, the combination agent is one of Lys^{B29}(-
30 myristoyl)des(B30) human insulin, Lys^{B29}(-tetradecanoyl)des(B30) human insulin and B²⁹-N -(N-lithocoly- -glutamyl)-des(B30) human insulin. Also

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suitable for combination therapy are non-peptide antihyperglycemic agents, antihyperlipidemic agents, and the like such as those well-known in the art.

In one embodiment, the invention encompasses methods of treating diabetes or related syndromes comprising administering a first amount of peptide S597 or peptide S557 and a second amount of a long-acting insulin analogue, such as, e.g., Lys^{B29}(-myristoyl)des(B30) human insulin, Lys^{B29}(-tetradecanoyl)des(B30) human insulin, or B²⁹-N -(NHthiocetyl- -glutamyl)-des(B30) human insulin, wherein the first and second amounts together are effective for treating the syndrome. As used herein, a long-acting insulin analogue is one that exhibits a protracted profile of action relative to native human insulin, as disclosed, e.g., in U.S. Patent Serial No. 6,451,970.

K. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Preferably, a pharmaceutical composition includes, in admixture, a pharmaceutically (i.e., physiologically) acceptable carrier, excipient, or diluent, and one or more of an IR or IGF-1R agonist or antagonist peptide, as an active ingredient. The preparation of pharmaceutical compositions that contain peptides as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically (i.e., physiologically) acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary

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substances such as wetting or emulsifying agents, pH-buffering agents, which enhance the effectiveness of the active ingredient.

An IR or IGF-1R agonist or antagonist peptide can be formulated into a pharmaceutical composition as neutralized physiologically acceptable salt forms. Suitable salts include the acid addition salts (i.e., formed with the free amino groups of the peptide molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutical compositions can be administered systemically by oral or parenteral routes. Non-limiting parenteral routes of administration include subcutaneous, intramuscular, intraperitoneal, intravenous, transdermal, inhalation, intranasal, intra-arterial, intrathecal, enteral, sublingual, or rectal. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration.

Intravenous administration, for example, can be performed by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., liquid used to dilute a concentrated or pure substance (either liquid or solid), making that substance the correct (diluted) concentration for use. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or

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oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e., blood) of the recipient.

Excipients suitable for use are water, phosphate buffered saline, pH 5 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or quantities, as well as routes of administration, used are 10 determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

Pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be 15 treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of IR or IGF-1R activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are specific for each individual. However, suitable dosages may range from about 10 to 200 nmol active 20 peptide per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous 25 infusions sufficient to maintain picomolar concentrations (e.g., approximately 1 pM to approximately 10 nM) in the blood are contemplated. An exemplary formulation comprises the IR or IGF-1R agonist or antagonist peptide in a mixture with sodium busulfite USP (3.2 mg/ml); disodium edetate USP (0.1 mg/ml); and water for injection q.s.a.d. (1 ml).

30 Further guidance in preparing pharmaceutical formulations can be found in, e.g., Gilman et al. (eds), 1990, *Goodman and Gilman's: The*

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Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, PA; Avis *et al.* (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman *et al.* (eds), 1990,

5 *Pharmaceutical Dosage Forms: Disperse Systems*, Dekker, New York.

The present invention further contemplates compositions comprising an IR or IGF-1R agonist or antagonist peptide, and a physiologically acceptable carrier, excipient, or diluent as described in detail herein.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. The amino acid sequences of the present invention can be used for gene therapy and thereby provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and non-viral transfer methods. A number of viruses have been used as gene transfer vectors, including polyoma, *i.e.*, SV40 (Madzak *et al.*, 1992, *J. Gen. Virol.*, **73**:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:39-6; Berkner *et al.*, 1988, *Bio Techniques*, **6**:616-629; Gorziglia *et al.*, 1992, *J. Virol.*, **66**:4407-4412; Quantin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, **89**:2581-2584; Rosenfeld *et al.*, 1992, *Cell*, **68**:143-155; Wilkinson *et al.*, 1992, *Nucl. Acids Res.*, **20**:2233-2239; Stratford-Perricaudet *et al.*, 1990, *Hum. Gene Ther.*, **1**:241-256), vaccinia virus (Mackett *et al.*, 1992, *Biotechnology*, **24**:495- 499), adeno-associated virus

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(Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.* **158**:91- 123; Ohi et al., 1990, *Gene*, **89**:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.* **158**:67-90; Johnson et al., 1992, *J. Virol.*, **66**:2952-2965; Fink et al., 1992, *Hum. Gene Ther.* **3**:11-19; Breakfield et al., 1987, *Mol. Neurobiol.*, **1**:337-371; Fresse et al., 1990, *Biochem. Pharmacol.* **40**:2189-2199), and retroviruses of avian (Brandyopadhyay et al., 1984, *Mol. Cell Biol.*, **4**:749-754; Petropoulos et al., 1992, *J. Virol.*, **66**:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.* **158**:1-24; Miller et al., 1985, *Mol. Cell Biol.*, **5**:431-437; Sorge et al., 1984, *Mol. Cell Biol.*, **4**:1730-1737; Mann et al., 1985, *J. Virol.*, **54**:401-407), and human origin (Page et al., 1990, *J. Virol.*, **64**:5370-5276; Buchschalcher et al., 1992, *J. Virol.*, **66**:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

Non-viral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham et al., 1973, *Virology*, **52**:456-467; Pellicer et al., 1980, *Science*, **209**:1414-1422), mechanical techniques, for example microinjection (Anderson et al., 1980, *Proc. Natl. Acad. Sci. USA*, **77**:5399-5403; Gordon et al., 1980, *Proc. Natl. Acad. Sci. USA*, **77**:7380-7384; Brinster et al., 1981, *Cell*, **27**:223-231; Constantini et al., 1981, *Nature*, **294**:92-94), membrane fusion-mediated transfer via liposomes (Felgner et al., 1987, *Proc. Natl. Acad. Sci. USA*, **84**:7413-7417; Wang et al., 1989, *Biochemistry*, **28**:9508-9514; Kaneda et al., 1989, *J. Biol. Chem.*, **264**:12126-12129; Stewart et al., 1992, *Hum. Gene Ther.* **3**:267-275; Nabel et al., 1990, *Science*, **249**:1285-1288; Lim et al., 1992, *Circulation*, **83**:2007-2011; U.S. Patent Nos. 5,283,185 and 5,795,587), and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990, *Science*, **247**:1465-1468; Wu et al., 1991, *BioTechniques*, **11**:474-485; Zenke et al., 1990, *Proc. Natl. Acad. Sci. USA*, **87**:3655-3659; Wu et al., 1989, *J. Biol. Chem.*, **264**:16985-16987; Wolff et al., 1991, *BioTechniques*, **11**:474-485; Wagner et al., 1991, *Proc. Natl. Acad. Sci. USA*, **88**:4255-4259; Cotten et al.,

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1990, *Proc. Natl. Acad. Sci. USA*, **87**:4033-4037; Curiel et al., 1991, *Proc. Natl. Acad. Sci. USA*, **88**:8850-8854; Curiel et al., 1991, *Hum. Gene Ther.* **3**:147-154).

Many types of cells and cell lines (e.g., primary cell lines or 5 established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g., myelocytes, macrophages, or monocytes), immune system cells of 10 different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g., K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the 15 foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary cells at various stages of development, 20 including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

In one approach, plasmid DNA is complexed with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular 25 complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

In another approach, liposome/DNA is used to mediate direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer 30 process is non-specific, localized *in vivo* uptake and expression have been

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reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992, *Hum. Gene Ther.* 3:399-410).

Suitable gene transfer vectors possess a promoter sequence, preferably a promoter that is cell-specific and placed upstream of the sequence to be expressed. The vectors may also contain, optionally, one or more expressible marker genes for expression as an indication of successful transfection and expression of the nucleic acid sequences contained in the vector. In addition, vectors can be optimized to minimize undesired immunogenicity and maximize long-term expression of the desired gene product(s) (see Nabe, 1999, *Proc. Natl. Acad. Sci. USA* 96:324-326). Moreover, vectors can be chosen based on cell-type that is targeted for treatment.

Illustrative examples of vehicles or vector constructs for transfection or infection of the host cells include replication-defective viral vectors, DNA virus or RNA virus (retrovirus) vectors, such as adenovirus, herpes simplex virus and adeno-associated viral vectors. Adeno-associated virus vectors are single stranded and allow the efficient delivery of multiple copies of nucleic acid to the cell's nucleus. Preferred are adenovirus vectors. The vectors will normally be substantially free of any prokaryotic DNA and may comprise a number of different functional nucleic acid sequences. An example of such functional sequences may be a DNA region comprising transcriptional and translational initiation and termination regulatory sequences, including promoters (e.g., strong promoters, inducible promoters, and the like) and enhancers which are active in the host cells. Also included as part of the functional sequences is an open reading frame (polynucleotide sequence) encoding a protein of interest. Flanking sequences may also be included for site-directed integration. In some situations, the 5'-flanking sequence will allow homologous recombination, thus changing the nature of the transcriptional initiation region, so as to provide for inducible or non-inducible transcription to increase or decrease the level of transcription, as an example.

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In general, the encoded and expressed peptide may be intracellular, i.e., retained in the cytoplasm, nucleus, or in an organelle, or may be secreted by the cell. For secretion, a signal sequence may be fused to the peptide sequence. As previously mentioned, a marker may be present for 5 selection of cells containing the vector construct. The marker may be an inducible or non-inducible gene and will generally allow for positive selection under induction, or without induction, respectively. Examples of marker genes include neomycin, dihydrofolate reductase, glutamine synthetase, and the like. The vector employed will generally also include an origin of 10 replication and other genes that are necessary for replication in the host cells, as routinely employed by those having skill in the art. As an example, the replication system comprising the origin of replication and any proteins associated with replication encoded by a particular virus may be included as part of the construct. The replication system must be selected so that the 15 genes encoding products necessary for replication do not ultimately transform the cells. Such replication systems are represented by replication-defective adenovirus (see G. Acsadi et al., 1994, *Hum. Mol. Genet.* 3:579-584) and by Epstein-Barr virus. Examples of replication defective vectors, particularly, retroviral vectors that are replication 20 defective, are BAG, (see Price et al., 1987, *Proc. Natl. Acad. Sci. USA*, 84:156; Sanes et al., 1986, *EMBO J.*, 5:3133). It will be understood that the final gene construct may contain one or more genes of interest, for example, a gene encoding a bioactive metabolic molecule. In addition, cDNA, synthetically produced DNA or chromosomal DNA may be employed 25 utilizing methods and protocols known and practiced by those having skill in the art.

According to one approach for gene therapy, a vector encoding an IR or IGF-1R agonist or antagonist peptide is directly injected into the recipient cells (*in vivo* gene therapy). Alternatively, cells from the intended recipients 30 are explanted, genetically modified to encode an IR or IGF-1R agonist or antagonist peptide, and reimplanted into the donor (*ex vivo* gene therapy).

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An ex vivo approach provides the advantage of efficient viral gene transfer, which is superior to in vivo gene transfer approaches. In accordance with ex vivo gene therapy, the host cells are first transfected with engineered vectors containing at least one gene encoding an IR or IGF-1R agonist or 5 antagonist peptide, suspended in a physiologically acceptable carrier or excipient such as saline or phosphate buffered saline, and the like, and then administered to the host or host cells. The desired gene product is expressed by the injected cells, which thus introduce the gene product into the host. The introduced gene products can thereby be utilized to treat or 10 ameliorate a disorder that is related to altered insulin or IGF-1 levels (e.g., diabetes).

The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the 15 vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The 20 amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

EXAMPLES

25 The examples as set forth herein are meant to exemplify the various aspects of the present invention and are not intended to limit the invention in any way.

The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Minneapolis, MN; Cat. # 30 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996.

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The insulin was either from Sigma (St. Louis, MO; Cat. # I-0259) or Boehringer. The IGF-1 was from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at 5 >80% purity. The Maxisorb Plates were from NUNC via Fisher (Cat. # 12565347). The HRP/Anti-M13 conjugate was from Pharmacia (Cat. # 27-9421-01). The ABTS solution was from BioF/X (Cat. # ABTS-0100-04).

Example 1: Monomer and Dimer Peptides

A. Cloning

10 Monomer and dimer peptides were constructed and expressed as protein fusions to a chitin binding domain (CBD) using the pTYB2 vector from the IMPACT™-CN system (New England Biolabs (NEB), Beverly, MA). The pTYB2 vector encodes a protein-splicing element (termed intein), which initiates self-cleavage upon the addition of DTT. The intein self-cleavage 15 separates the dimer from the affinity tag, to allow purification.

In the pTYB2 construct, the C-terminus of the peptide sequence was fused to the N-terminus of the intein/CBD sequence. Two peptide-flanking epitope tags were included: a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. This fusion was generated by ligating a vector 20 fragment encoding the intein/CBD with a PCR product encoding the peptide of interest.

The vector fragment was obtained by digesting at appropriate restriction sites the pTBY2 vector. The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN, 25 Valencia, CA). To obtain the PCR product of the target proteins, primers were synthesized which anneal to appropriate sequences. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were

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performed at 1500 V in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 μ l of *E. coli* strain BL21.

Immediately following electroporation, 1 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added to the
5 transformants. The transformants were grown at 37°C for 1 h, and then plated onto 2xYT-AG plates and incubated overnight at 37°C. Individual colonies were isolated and used to inoculate 2xYT-G. The cultures were grown overnight at 37°C. Plasmid DNA was isolated from the cultures and sequencing was performed to confirm that the correct construct was
10 obtained.

B. Small-scale expression of peptide-CBD fusion proteins

E. coli ER2566 (New England Biolabs) containing plasmids encoding peptide-CBD fusion proteins were grown in 2xYT-AG at 37°C overnight, with agitation (250 rpm). The following day, the cultures were used to inoculate
15 media (2x YT-G) to obtain an OD₆₀₀ of 0.1. Upon reaching an OD₆₀₀ of 0.6, expression of the fusion protein was induced by the addition of IPTG (isopropyl- β -D-thiogalactopyranoside) to a final concentration of 0.3 mM. Cells were grown for 3 h. Following this, cells were pelleted by centrifugation and the cell pellets were analyzed by SDS-PAGE
20 electrophoresis. Production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Amersham Pharmacia).

C. Large-scale expression and purification of soluble peptide-CBD fusion proteins

25 *E. coli* ER2566 carrying plasmids encoding the fusion proteins were grown in 2xYT-AG media at 37°C for 8 h, with agitation (250 rpm). The cultures were back-diluted into to 2 L volumes of 2xYT-A to achieve an OD₆₀₀ of 0.1. Upon reaching an OD₆₀₀ of 0.5, IPTG was added to a final concentration of 0.3 mM. Cells were grown at 30°C overnight. The next day
30 cells were isolated by centrifugation. Samples of the cell pellet were

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analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

5 The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent. After removal of cell debris by centrifugation, the soluble proteins in the clarified lysate were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The CBD fusions were purified by chitin affinity
10 chromatography according to the manufacturer's instructions (New England Biolabs). The lysate was loaded onto a chitin affinity column and the column was washed with 10 volumes of column buffer. Three bed volumes of the DTT containing cleavage buffer were loaded onto the column and the column was incubated overnight. The next day, the target protein was
15 eluted by continuing the flow of the cleavage buffer without DTT. The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

Example 2: PEG-Based Dimer Peptides

A. Synthesis of the aldehyde containing peptide

20 The peptide was synthesized by stepwise solid phase synthesis on Rink amide Tentagel (0.21 mmol/g). Three equivalents of Fmoc-amino acids were used. The serine residue was introduced into the peptide by either coupling Fmoc-Ser(tBu)-OH to the N-terminal peptide or coupling Boc-Ser(tBu) to a selectively protected lysine side-chain. The peptide was
25 then deprotected and cleaved from the resin by treatment with 95% TFA (trifluoroacetic acid; aq) containing TIS (triisopropylsilan). Periodate oxidation, using 2 equivalent of NaIO₄ in 20% DMSO (dimethyl sulfoxide)-80% phosphate buffer pH 7.5 (45 µl/µmol peptide) for 5 min at room

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temperature (RT), converted the 2-amino alcohol moiety in an α -oxoacyl group. The peptide was purified immediately following oxidation.

B. Synthesis of the PEG-based dimer

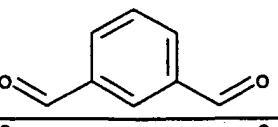
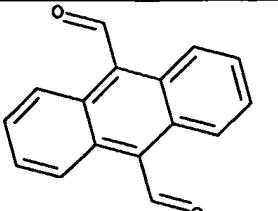
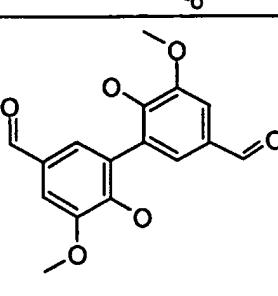
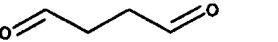
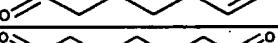
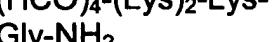
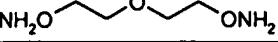
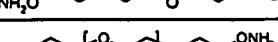
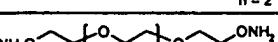
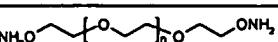
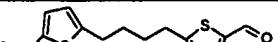
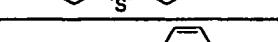
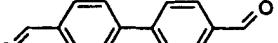
The unprotected and oxidized peptide (4.2 equivalent) was dimerized 5 on the dioxyamino-PEG (polyethylene glycol)-linker (1 equivalent) in 90% DMSO-10% 20 mM NaOAc buffer, pH 5.1 (4.2 μ l/ μ mol peptide). The solution was left for 1 hr at 38°C and the progress of the reaction was monitored by MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry). Following this, the crude dimer was purified by semi-10 preparative HPLC (high performance liquid chromatography).

The molecular weights and inter peptide distance of various linkers is shown in Table 3, below.

TABLE 3

Structure	Number	MW	MW (- 2H₂O)
	1	100.1	64.1
	2	58.04	22.04
	3	149.15	113.15
	4	150.14	114.14
	5	134.13	98.13

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	6	134.13	98.13
	7	134.13	98.13
	8	234.25	198.25
	9	302.3	266.3
	10	72.06	36.06
	11	86.09	50.09
	12	114.14	78.14
	13	128.08	92.08
	14	142.19	106.19
(HCO) ₄ -(Lys) ₂ -Lys-Gly-NH ₂	15		
	16	136.2	100.2
	17	180.2	144.2
	18	224.3	188.3
	19	268.3	232.3
	20	312.4	276.4
	21	278.4	242.4
	22	240.3	204.3
	23	240.3	204.3
	24	210.2	192.2

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Example 3: Determination of Insulin Receptor Binding

IR was incubated with ^{125}I -labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from 5 transfected cells after solubilization with Triton X-100. The assay buffer contained 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MgCl₂, 0.5% human serum albumin (HSA), 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 10 2000 cpm (3 pM) of its ^{125}I -labeled ligand (TyrA14- ^{125}I -HI or Tyr31- ^{125}I -IGF1) and a dilution series of the substance to be tested was added. After equilibration for 2 days at 4°C, each sample (200 μl) was precipitated by addition of 400 μl 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

15 The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin competition are shown in Figures 10A-10C; 11A-11D. Qualitative data are provided in Table 4, below.

20 Table 4 illustrates IR affinities for the RP9 monomer peptide and various RP9 monomer truncations. The results demonstrate that RP9 N-terminal sequence (GSLD; SEQ ID NO:1785) and C-terminal sequence (LGKK; SEQ ID NO:1786) can be deleted without substantially affecting HIR binding affinity (Table 4).

25

TABLE 4

Peptide	SEQ ID NO:	Formula	Site IR	Sequence	HIR Kd (mol/l)
S386	1559	1	1	GSLDESFYDWFERQLG	3.2×10^{-7}
S395	1787	1	1	GSLDESFYDWFERQL	9.1×10^{-8}
S394	1788	1	1	GSLDESFYDWFERQ	8.1×10^{-8}
S396	1789	1	1	GSLDESFYDWFER	$>2 \times 10^{-5}$
S399	1790	1	1	ESFYDWFERQL	9.1×10^{-8}
S400	1791	1	1	ESFYDWFERQ	6.3×10^{-7}

Figures 10A-10C demonstrate that Site 1-Site 2 heterodimer peptides 537, 538, and 539 bound to IR with substantially higher (several orders of magnitude) affinity than corresponding monomer (D117 and 540) and 5 homodimer (521 and 535) peptides. Figures 11A-11D demonstrate that Site 1-Site 2 heterodimer peptides, 537 and 538, bound to IR with markedly higher affinity than the monomer peptide D117.

Example 4: Adipocyte Assay for Determination of Insulin Agonist Activity

10 Insulin increases uptake of ^3H glucose into adipocytes and its conversion into lipid. Incorporation of ^3H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^3H products. The effect of compounds on the incorporation of ^3H glucose at a sub-maximal insulin dose was determined, and the results 15 expressed as increase relative to full insulin response. The method was adapted from Moody *et al.*, 1974, *Horm Metab Res.* 6(1):12-6.

Mouse epididymal fat pads were dissected out, minced into digestion buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and digested for up to 1.5 h at 36.5°C. After 20 filtration, washing (Krebs-Ringer HEPES, 1% HSA), and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), free fat cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED₂₀ insulin.

The assay was started by addition of ^3H glucose (Amersham TRK 25 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard 30 Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate.

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Data are presented graphically, as effect of compound on an (approximate) ED₂₀ insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1
5 are shown in Figures 12-18. Qualitative data are shown in Tables 5-7.

In free fat cell (FFC) assays, truncated synthetic RP9 monomer peptides S390 and S394 showed potency similar to full-length RP9 monomer peptides (Figures 12A-12D). Truncated synthetic RP9 homodimer peptides S415 and S417 were highly potent in FFC assays, but less potent
10 than full-length RP9 homodimer peptides (Figures 13A-13C; compare to peptides 521 and 535, described below). The potency of recombinant RP9 homodimer peptides 521 and 535 in FFC assays is shown in Figures 14A-14C. The curves are flattened, suggesting that the binding mechanism may not be mediated by simple intramolecular binding (Figures 14A-14C).

15 Results further indicated that synthetic RP9 homodimer peptides S337 and S374 showed increased HIR biding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371 (Table 5). Similarly, synthetic RP9 homodimer peptides S314 and S317 showed increased HIR binding affinity and increased potency in FFC assays
20 compared to synthetic RP9 monomer, S371, and various RP9 truncations (Table 6).

TABLE 5

Pep.	SEQ ID NO:	Formula	Site IR	Monomer or Dimer	Sequence	HIR K _d (mol/l)	FFC
S371	1558	1	1	M (RP9)	GSLDESFYDWFERQLGKK	6.3*10 ⁻⁷	+
S337	1792	1-1	1-1	D, C-Term 23	(GSLDESFYDWFERQLGKK-Lig) ₂ -23	1.1*10 ⁻⁸	++++
S374	1793	1-1	1-1	D, N-Term 17	17-(GSLDESFYDWFERQLGKK) ₂	1.8*10 ⁻⁷	+++

25 M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 and 17 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist.

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TABLE 6

Peptide	SEQ ID NO:	Form.	Site IR	Mon. or Dimer	Sequence	HIR K _d (mol/l)	FFC
S371 (RP9)	1558	1	1	M	GSLDESFYDWFERQLGKK	6.3.*10 ⁻⁷	+
S395	1787	1	1	M	GSLDESFYDWFERQL	9.1*10 ⁻⁸	+
S394	1788	1	1	M	GSLDESFYDWFERQ	8.1*10 ⁻⁸	++
S396	1789	1	1	M	GSLDESFYDWFER	>2*10 ⁻⁵	0
S390	1794	1	1	M	ESFYDWFERQLG	6.2*10 ⁻⁷	+
S399	1790	1	1	M	ESFYDWFERQL	9.1*10 ⁻⁸	++
S400	1791	1	1	M	ESFYDWFERQ	6.3*10 ⁻⁷	0
S415	1795	1-1	1-1	D; C-Term	(ESFYDWFERQLGK) ₂ -23	1.0*10 ⁻⁷	++++
S417	1796	1-1	1-1	D; N-Term	23-(ESFYDWFERQLG) ₂	9.2*10 ⁻⁷	+++

5 M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 represents a specific chemical linker (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist; Form. = formula; Mon. = monomer;

10 Site 1-Site 2 dimer peptides 537 and 538 were inactive in the FFC assays using the standard concentration of insulin (Figures 15A-15C). However, Site 1-Site 2 dimer peptides 537 and 538 were antagonists in the FFC assay in the presence of a stimulating concentration of insulin (Figures 16A-16C). In contrast, Site 2-Site 1 dimer peptide 539 was a full agonist in the FFC assay, with a slope similar to that of insulin (Figures 17A-17B).

15 Additional experiments confirmed that FFC assay activity of Site 1-Site 2 dimer peptides was affected by the orientation of the monomer subunits (Figures 18A-18D). In particular, dimer peptides comprising Site 1 (S372 or S373) and Site 2 (S451 or S452) monomer subunits exhibited antagonist activity in the Site 1-Site 2 orientation (C-N linkage) (dimer peptide S453); moderate levels of agonist activity in the Site 1-Site 2 orientation (N-N or C-C linkage) (dimer peptides S454 and S456); and high 20 levels of agonist activity in the Site 2-Site 1 orientation (C-N linkage) (dimer peptide S455) (Figures 18A-18D).

Table 7, below, shows the HIR binding affinity and FFC assay potency of various synthetic peptides, including Site 1-Site 1 dimer peptides S325, S329, S332; S333, S334, S335, S336, S337, S349, S350, S351,

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S352, S353, S354, S361, S362, S363, S374, S375, S376, S378, S379, S380, S381, S414, S415, S416, S417, S418, S420, and S424. These synthetic dimer peptides exhibited properties comparable to dimer peptides 521 and 535, regardless of the orientation of the monomer subunits. In 5 particular, synthetic Site 1-Site 2 dimer peptides S425, S453, and S459 exhibited antagonist properties comparable to those of the Site 1-Site 2 dimer peptides 537 and 538. Synthetic Site 1-Site 2 dimer peptides S455, S457, and S458 exhibited agonist properties comparable to the dimer peptide 539. Synthetic Site 1-Site 2 dimer peptides S436, S437, S438, 10 S454, S456 act as partial agonists in the FFC assay (i.e., the peptides exhibit a maximal response of less than 100% that of insulin), which is shown in the table as “++” and “+++”.

Table 7 also shows properties of truncated monomer and dimer peptides, and thereby indicates which N- or C-terminal residues can be 15 deleted without substantial loss of HIR binding affinity (e.g., see synthetic peptides S386 through S392, S394 through S403, and S436 through S445). Notably, certain Site 2-Site 1 dimers show IR affinities of 2×10^{-11} (see, e.g., S519 and S520). These peptides are also very potent in the fat cell assay (Figures 31A-31B) and even more potent in the HIR kinase assay (Figures 20 32A-32B) (kinase assay described below).

TABLE 7

Peptide	SEQ ID NO:	Formula	Linkage	Site IR	Sequence	HIR Kd (mol/l)	FFC
S105	1797	F1	-	1	FHENFYDWFRVRQVAKK	3.1*10 ⁻⁷	++
S106	1798	F1	-	1	FHENFYDWFRVRQASKK	4.2*10 ⁻⁷	++
S107	1799	F1	-	1	FHENFYDWFRVRAVSKK	10.0*10 ⁻⁷	+
S108	1800	F1	-	1	FHENFYDWFRVAQVSKK	7.5*10 ⁻⁷	+
S109	1801	F1	-	1	FHENFYDWFRARQVSKK	2.3*10 ⁻⁷	++
S110	1802	F1	-	1	FHEAFYDWFRVRQVSKK	2.2*10 ⁻⁷	++
S111	1803	F1	-	1	FHANFYDWFRVRQVSKK	3.3*10 ⁻⁷	0
S112	1804	F1	-	1	FAENFYDWFRVRQVSKK	6.1*10 ⁻⁷	+
S113	1805	F1	-	1	AHENFYDWFRVRQVSKK	5.9*10 ⁻⁷	+
S114	1556	F1	-		fhemfydwfrvrqvskk	8.3*10 ⁻⁶	0
S115	1806	F1	-	1	EHHENFYDWFRVRQVSEE	6.5*10 ⁻⁷	+
S116	1807	F1	-	1	FHENFYGWFRVRQVSKK	1.4*10 ⁻⁶	++
S117	1808	F2	-	1	HETFYSMIRSLAK	2.7*10 ⁻⁶	0
S118	1809	F2	-	1	SDGFYNAIELLS	2.4*10 ⁻⁶	+
S119	1810	F2	-	1	SLNFYDALQLLAKK	1.8*10 ⁻⁶	0
S120	1811	F2	-	1	HDFFYSMMKSLK	2.0*10 ⁻⁶	0
S121	1812	F2	-	1	NSFYEARMLSSK	3.1*10 ⁻⁶	0
S122	1813	F7	-		HPTSKKEYAKLLK	9.3*10 ⁻⁶	0
S123	1814	F7	-		HPSTNQMLMKLFK	1.6*10 ⁻⁵	0
S124	1815	F7	-		HPPLSEKLFLIKK	2.3*10 ⁻⁵	0
S127	1816	F2	-	1	WSDFYSYFQGLD	1.2*10 ⁻⁶	0
S128	1817 and 1818	F1-F1	C-C	1-1	(FHENFYDWFRVRQVSKK)-Dap	1.1*10 ⁻⁶	++
S129	1819	F2	-	1	SSNFYQALMLLS	2.9*10 ⁻⁶	0
S131	1820	F1	-	1	FHENFYDWFRVRQVSKK-Lig	1.2*10 ⁻⁶	+

S137	1821	F1	-	1	HENFYGWFVRQVSKK (FHENFYDWFWRQVSKK)2-Lys	7.7*10 ⁻⁷	0
S145	1822 and 1823	F1-F1	C-C	1-1		1.5*10 ⁻⁶	++
S158	1780	F1	-	1	FHENFYDWFWRQVSK	8.1*10 ⁻⁷	+
S165	1554	F1	-	1	FYDWF	>2*10 ⁻⁵	0
S166	1824	F1	-	1	FYDWFKK	>2*10 ⁻⁵	0
S167	1825	F1	-	1	AFYDWFAKK	>2*10 ⁻⁵	-
S168	1826	F1	-	1	AAAAYDWFAAAAAKK	3.8*10 ⁻⁶	0
S169	1827 and 1828	F1-F1	N-N	1-1	12-(Lig-FHENFYDWFWRQVSKK)2 (CGFHENFYDWFWRQVSKK)2 (linked at cysteines)	5.8*10 ⁻⁷	++
S170	1829 and 1830	F1-F1	N-N	1-1		7.0*10 ⁻⁷	+++
S171	1831	F1	-	1	CGFHENFYDWFWRQVSKK	2.9*10 ⁻⁶	+++
S172	1832 and 1833	F1-F1	N-N	1-1	14-(Lig-FHENFYDWFWRQVSKK)2	4.8*10 ⁻⁶	+++
S173	1834	F3	-	1	LDALDRLMRYFEERPSL	1.2*10 ⁻⁶	0
S174	1835	F3	-	1	PLAELWAYFEHSEQGRSSAH	1.6*10 ⁻⁵	0
S175	1560	F1	-	1	GRVDWLQRNANFYDWFWAELG	2.3*10 ⁻⁷	+++
S176	1836	F1	-	1	NGVERAGTGDNFYDWFAQLH	4.7*10 ⁻⁷	+
S177	1837	F2	-	1	EHWNTVDPFYFTLFEWLRESG	2.7*10 ⁻⁶	0
S178	1838	F2	-	1	EHWNTVDPFYQYFSELLRESG	1.3*10 ⁻⁷	++
S179	1839	F1	-	1	QSDSGTVHDIFYGWFRDTWAS	5.4*10 ⁻⁷	+
S180	1840	F1	-	1	AFYDWFAK	>2*10 ⁻⁵	0
S181	1841	F1	-	1	AFYDWF	>2*10 ⁻⁵	0
S182	1842	F1	-	1	AFYDWF	>2*10 ⁻⁵	0
S183	1843	F1	-	1	FYDWF	>2*10 ⁻⁵	0
S184	1844	F1	-	1	Ac-FYDWF	>2*10 ⁻⁵	0
S214	1845	F1	-	1	AFYEWFAKK	>2*10 ⁻⁵	0
S215	1846	F1	-	1	AFYGWFAKK	>2*10 ⁻⁵	0
S216	1847	F1	-	1	AFYKWFAKK	>2*10 ⁻⁵	0
S217	1848 and 1849	F2-F2	C-C	1-1	(SDGFYNAIELLS(Lig))2-14	3.9*10 ⁻⁸	++
S218	1850 and 1851	F1-F1	C-C	1-1	(AFYDWFAKK-Lig)2-14	1.1*10 ⁻⁵	0

S219	1852	F1	-	1	FHENAYDWFRQRVSKK	>2*10 ⁻⁵	0
S220	1853	F1	-	1	FHENFADWFVRQVSKK	>2*10 ⁻⁵	0
S221	1854	F1	-	1	FHENFYAWFVRQVSKK	1.1*10 ⁻⁶	+
S222	1855	F1	-	1	FHENFYDAFWRQVSKK	>2*10 ⁻⁵	0
S223	1856	F1	-	1	FHENFYDWAVRQVSKK	>2*10 ⁻⁵	0
S226	1857	F6	-	2	QLEEEWAGVQCEVYGRECPSSDGFYNAIELLS-Lig	1.6*10 ⁻⁶	
S227	1858	F1	-	1	CGGFHENFYDWFRQVSKK	5.1*10 ⁻⁷	++
S228	1859 and 1860	F1-F1	N-N	1-1	(CGGFHENFYDWFRQVSKK) ₂ (linked at cysteines)	3.6*10 ⁻⁷	++
S229	1861 and 1862	F2-F4	C-C	1-2	KHLCVLEELFWGASLFGYCSGKK-Lig	4.4*10 ⁻⁹	0
S231	1863 and 1864	F1-F1	C-C	1-1	(FHENFYDWFRQVSKKGGG-Lig) ₂ -14	2.7*10 ⁻⁷	+
S232	1865 and 1866	F1-F1	N-N	1-1	14-(Lig-GGGFHENFYDWFRQVSKK) ₂	3.8*10 ⁻⁷	+++
S233	1867 and 1868	F1-F2	C-C	1-1	FHENFYDWFRQVSKK-Lig	2.6*10 ⁻⁷	+
S234	1869	F1	-	1	SDGFYNAIELLS-Lig	1.3*10 ⁻⁷	++
S235	1870	F1	-	1	RVDWLQRNANFYDWVAELG	5.3*10 ⁻⁸	++
S236	1871	F1	-	1	DWLQRNANFYDWVAELG	1.0*10 ⁻⁷	++
S237	1872	F1	-	1	WLQRNANFYDWVAELG	8.5*10 ⁻⁷	0
S238	1873	F1	-	1	LQRNANFYDWVAELG	8.5*10 ⁻⁷	0
S239	1874	F1	-	1	QRNANFYDWVAELG	1.3*10 ⁻⁶	0
S240	1875	F1	-	1	RNANFYDWVAELG	1.4*10 ⁻⁶	
S241	1876	F1	-	1	NANFYDWVAELG	1.6*10 ⁻⁶	
S242	1877	F1	-	1	ANFYDWVAELG	2.0*10 ⁻⁶	
S243	1878	F1	-	1	NFYDWVAELG	2.0*10 ⁻⁶	
S244	1879	F1	-	1	GRWDWLQRNANFYDWVAELG-Lig	2.2*10 ⁻⁷	++
S245	1880	F1	-	1	Lig-GRWDWLQRNANFYDWVAELG	2.2*10 ⁻⁷	+

S246	1881 and 1882	F8-F1	C-C	3-1	ACAWPTYWNICGGG-Lig 14	5.0*10 ⁻⁶	
					FHENFYDWLVRQVSKK-Lig		
S248	1883	F1	-	1	GRVDWLQRNANFYDWFWAEL	6.3*10 ⁻⁸	++
S249	1884	F1	-	1	GRVDWLQRNANFYDWFWAE	7.4*10 ⁻⁷	0
S250	1885	F1	-	1	GRVDWLQRNANFYDWFWA	8.9*10 ⁻⁶	0
S251	1886	F1	-	1	GRVDWLQRNANFYDWFWV	5.6*10 ⁻⁶	
S252	1887 and 1888	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig)z-14	4.4*10 ⁻⁷	0
S253	1889 and 1890	F1-F1	C-C	1-1	(GRVDWLQRNANFYDWFWAELG-Lig)z-14	2.2*10 ⁻⁸	++
S255	1891 and 1892	F2-F2	C-C	1-1	(SDGFYNAIELLSGGG-Lig)z-14	1.6*10 ⁻⁶	0
S256	1893	F6	-	2	Acy-CLEEWGASL-Tic-QCSG	9.0*10 ⁻⁶	-
S257	1894	F2	-	1	RWPNFYGYFESILTHFS	1.4*10 ⁻⁵	0
S259	1895	F2	-	1	EGWDFYSYFSGLLASVT	7.7*10 ⁻⁶	0
S260	1896	F2	-	1	LDRQFYRYFQDLLVGFM	2.3*10 ⁻⁶	0
S261	1897	F2	-	1	WGRSFYRYFETLLAQGI	>2*10 ⁻⁵	0
S262	1898	F4	-	1	PLCFLQELFGGASLGGYCSG	1.9*10 ⁻⁵	0
S263	1899	F6	-	2	WLEQERAWIWCIEIQGSGCRA	>2*10 ⁻⁵	0
S264	1900	F1	-	1	IQGWEFPYGWFDVVAQMFE	1.9*10 ⁻⁷	0
S265	1901	F1	-	1	TGHRLGLDEQFYWWFRDALSG	1.1*10 ⁻⁷	0
S266	1902	F6	-	2	Abu-CLEEWGASL-Tic-QCSG	>2*10 ⁻⁵	0
S268	1903	F1	-	1	RD-Hyp-FYDWFFDDI	4.5*10 ⁻⁷	0
S273	1904	F1-F2	C-N	1-1	FHENFYDWLVRQVSKK-Lig-14-Lig-SDGFYNAIELLS	1.5*10 ⁻⁶	+
S278	1905	F1-derived	-	1	GREGQRWYWFVAQVT	>2*10 ⁻⁵	0
S281	1906	F5	-		DLRVLCELFGAYVLGYCSE	1.1*10 ⁻⁵	0
S282	1907	F4-derived	-		HLSVGEELSMWVALLGQWAR	>2*10 ⁻⁵	0
S283	1908	F4-derived	-		APVSTEELRWGALLFGQWAG	>2*10 ⁻⁵	0
S284	1909	F6-derived	-		ALEEEWAWVQVRSIRSGLPL	>2*10 ⁻⁵	0
S285	1910	F6-derived	-		WLEHEWAQIQCELYGRGCTY	8.3*10 ⁻⁷	

S287	1911	F1	-	1	QAPSNFYDWFVREWDEE	5.9*10 ⁻⁶	0
S288	1912	F2	-	1	QSFYDYIEELGGGEWKK	4.3*10 ⁻⁶	0
S289	1913	F2	-	1	DPFYQQGLWEWLRESGEE	>2*10 ⁻⁵	0
S290.	1914 and 1915	F1-F1	N-N	1-1	7-(Lig-GGGHENFYDWFVRQVSKK) ₂	9.0*10 ⁻⁷	++
S291	1916 and 1917	F1-F1	N-N	1-1	9-(Lig-GGGHENFYDWFVRQVSKK) ₂	1.2*10 ⁻⁶	++++
S292	1918 and 1919	F1-F1	N-N	1-1	12-(Lig-GGGHENFYDWFVRQVSKK) ₂	7.5*10 ⁻⁷	++
S293	1920 and 1921	F1-F1	N-N	1-1	13-(Lig-GGGHENFYDWFVRQVSKK) ₂	1.2*10 ⁻⁷	++
S294	1922	F1	-	1	DWLQRNANFYDWFVAEL-Lig	1.3*10 ⁻⁷	++
S295	1923	F1	-	1	Lig-DWLQRNANFYDWFVAEL	4.8*10 ⁻⁷	+
S300	1924 and 1925	F1-F1	C-C	1-1	(DWLQRNANFYDWFVAEL-Lig') ₂ -14	5.0*10 ⁻⁸	+++
S301	1926 and 1927	F1-F1	N-N	1-1	14-(Lig'-DWLQRNANFYDWFVAEL) ₂	6.4*10 ⁻⁷	+
S302	1928	F2	-	1	SDGFYNA-Acy-ELLSG	8.6*10 ⁻⁷	0
S303	1929	F2	-	1	SGFPFYE-Acy-ELLWAib-G	5.7*10 ⁻⁶	0
S304	1930	F2	-	1	GGSFYDD-Acy-E-Aib-LW-Aib-G	2.1*10 ⁻⁵	0
S305	1931	F2	-	1	N-Aib-PFYDE-Acy-DE-Cha-W-Aib-G	8.4*10 ⁻⁷	0
S306	1932	F1	-	1	GRVDWLQRNANFYDWFVAE-Acy-G	2.2*10 ⁻⁶	+++
S312	1933 and 1934	F1-F1	N-N	1-1	23-(Lig-GGGHENFYDWFVRQVSKK) ₂	2.9*10 ⁻⁶	++
S313	1935 and 1936	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig') ₂ -23	2.4*10 ⁻⁷	
S315	1937	F1	-	1	WFYDWFWE	6.8*10 ⁻⁶	0
S316	1938	F10	-	1	WQGYAWLS	7.0*10 ⁻⁶	0
S317	1939	F10	-	1	WPGYAWLS	>2*10 ⁻⁵	0
S319	1940	F1	-	1	D-Aic-D-Aib-EFYDWFDEiGq	8.7*10 ⁻⁷	0
S320	1941	F1	-	1	KNNKEFYEWFDiGq	2.8*10 ⁻⁶	0
S321	1942	F1	-	1	YerD-Hyp-FYDWFDEiHyp-e	1.4*10 ⁻⁶	0
S322	1943	F1	-	1	EWRD-Hyp-FYDWFDEiHyp-e	7.2*10 ⁻⁷	0
S325	1944 and 1945	F1-F1	N-N	1-1	9-(Lig'-GSLDESFYDWFERQLGKK) ₂	4.6*10 ⁻⁸	++++
S326	1600	F1	-	1	GILSQSCPESFYDWFAGQQSDPWWCW	5.9*10 ⁻⁷	-
S327	1946	F2	-	1	TFYSCSCLSLTGTQPQPNRGPWERCRKK	2.1*10 ⁻⁶	-
S329	1947 and 1948	F1-F1	N-N	1-1	17-(Lig'-FHENFYDWFVRQVSKK) ₂	2.7*10 ⁻⁶	++
S331	1949	F4	-	2	KHLCVLEELFWGASLFGYCSEGKK	1.6*10 ⁻⁶	0
S332	1950 and 1951	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig') ₂ -9	2.1*10 ⁻⁸	++++

S333	1952 and 1953	F1-F1	N-N	1-1	22-(Lig'-GSLDESFYDWFERQLGKK) ₂	1.4*10 ⁻⁷	++++
S334	1954 and 1955	F1-F1	N-N	1-1	22-(Lig'-GGGFHENFYDWVRQVSKK) ₂	1.6*10 ⁻⁶	+++
S335	1956 and 1957	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig) ² -22	9.8*10 ⁻⁸	+++
S336	1958 and 1959	F1-F1	N-N	1-1	23-(Lig'-GSLDESFYDWFERQLGKK) ₂	1.5*10 ⁻⁸	+++
S337	1960 and 1961	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig) ² -23	1.1*10 ⁻⁸	++++
S342	1962	F1	-	1	DLWIFNAKEDMNFYDWFWNQLR	1.8*10 ⁻⁶	0
S344	1963	F2	-	1	EHWNNTVDPFYHWISELLRESGA	2.0*10 ⁻⁷	0
S345	1964	F2	-	1	EHWNNTVDPFYQYFAELLRESGA	2.9*10 ⁻⁶	0
S349	1965 and 1966	F1-F1	N-N	1-1	23-(Lig'-GGGFHENFYDWVRQVSKK) ₂	1.3*10 ⁻⁷	+++
S350	1967 and 1968	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig) ² -21	4.7*10 ⁻⁷	++++
S351	1969 and 1970	F1-F1	N-N	1-1	21-(Lig'-GSLDESFYDWFERQLGKK) ₂	1.4*10 ⁻⁶	+++
S352	1971 and 1972	F1-F1	N-N	1-1	21-(Lig'-GGGFHENFYDWVRQVSKK) ₂	6.6*10 ⁻⁷	+++
S353	1973 and 1974	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig) ² -14	1.1*10 ⁻⁸	+++++
S354	1975 and 1976	F1-F1	N-N	1-1	14-(Lig'-GSLDESFYDWFERQLGKK) ₂	3.9*10 ⁻⁸	++++
S359	1977 and 1978	F1-F1	N-N	1-1	9-(Lig'-DWLQRNANFYDWFWVAEL) ₂	7.0*10 ⁻⁷	+
S360	1979 and 1980	F1-F1	N-N	1-1	23-(Lig'-DWLQRNANFYDWFWVAEL) ₂	9.9*10 ⁻⁷	
S361	1981 and 1982	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig) ² -24	2.2*10 ⁻⁶	+++
S362	1983 and 1984	F1-F1	N-N	1-1	24-(Lig'-GSLDESFYDWFERQLGKK) ₂	1.1*10 ⁻⁷	++++
S363	1985 and 1986	F1-F1	N-N	1-1	24-(Lig'-GGGFHENFYDWVRQVSKK) ₂	2.2*10 ⁻⁷	+++
S365	1987	F1	-	1	RMYFSTGAPQNFYDWFWYQEWD	1.0*10 ⁻⁵	0
S366	1988	F1	-	1	PILRESRNFYDWFWYQOLE	3.7*10 ⁻⁷	0
S368	1989	F2	-	1	RGRSDPPFYHLSSELLQGH	>2*10 ⁻⁵	0
S371	1558	F1	-	1	GSLDESFYDWFERQLGKK	6.3.*10 ⁻⁷	+
S372	1990	F1	-	1	SGSDESFYDWFERQLGKK	2.0*10 ⁻⁷	++
S373	1991	F1	-	1	GSLDESFYDWFERQLGKK(S)	1.2*10 ⁻⁷	+++
S374	1992 and 1993	F1-F1	N-N	1-1	17-(Ad-GSLDESFYDWFERQLGKK)-17-(Ad-GSLDESFYDWFERQLGKK) ₂	1.8*10 ⁻⁷	++++
S375	1994	F1-F1	C-N	1-1	(GSLDESFYDWFERQLGKK-Ad)-14-(Ad-GSLDESFYDWFERQLGKK)	2.0*10 ⁻⁷	+++
S376	1995 and 1996	F1-F1	N-N	1-1	19-(Ad-GSLDESFYDWFERQLGKK) ₂	1.6*10 ⁻⁷	++++
S377	1997 and 1998	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Ad)-17	6.5*10 ⁻⁸	++++
S379	1999 and 2000	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Ad)-19	5.6*10 ⁻⁸	++++
S380	2001 and 2002	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFWVAEL-Lig) ² -9	5.1*10 ⁻⁷	++
S381	2003 and 2004	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFWVAEL-Lig) ² -23	1.2*10 ⁻⁷	++++

S386	1559	F1	-	1	GSLDESFYDWFERQLG	3.2*10 ⁻⁷	+
S387	2005	F1	-	1	SLDESFYDWFERQLG	6.3*10 ⁻⁷	+
S388	2006	F1	-	1	LDESFYDWFERQLG	3.4*10 ⁻⁷	+
S389	2007	F1	-	1	DESFYDWFERQLG	1.1*10 ⁻⁶	+
S390	1794	F1	-	1	ESFYDWFERQLG	6.2*10 ⁻⁷	+
S391	2008	F1	-	1	SFYDWFERQLG	1.5*10 ⁻⁶	+
S392	2009	F1	-	1	FYDWFERQLG	3.8*10 ⁻⁶	0
S394	1788	F1	-	1	GSLDESFYDWFERQ	9.1*10 ⁻⁸	+
S395	1787	F1	-	1	GSLDESFYDWFERQL	8.1*10 ⁻⁸	++
S396	1789	F1	-	1	GSLDESFYDWFER	>2*10 ⁻⁵	0
S397	2010	F1	-	1	GSLDESFYDWFE	>2*10 ⁻⁵	0
S398	2011	F1	-	1	GSLDESFYDWF	>2*10 ⁻⁵	0
S399	1790	F1	-	1	ESFYDWFERQL	9.5*10 ⁻⁸	++
S400	1791	F1	-	1	ESFYDWFERQ	6.3*10 ⁻⁷	0
S401	2012	F1	-	1	ESFYDWFER	>2*10 ⁻⁵	0
S402	2013	F1	-	1	ESFYDWFE	>2*10 ⁻⁵	0
S403	2014	F1	-	1	ESFYDWF	>2*10 ⁻⁵	0
S414	2015 and 2016	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig) ₂ -14	3.8*10 ⁻⁷	++++
S415	2017 and 2018	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig) ₂ -23	1.0*10 ⁻⁷	++++
S416	2019 and 2020	F1-F1	N-N	1-1	14-(Lig'-ESFYDWFERQLG) ₂	9.3*10 ⁻⁷	+++
S417	2021 and 2022	F1-F1	N-N	1-1	23-(Lig'-ESFYDWFERQLG) ₂	9.2*10 ⁻⁷	+++
S418	2023 and 2024	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Ad) ₂ -17	1.2*10 ⁻⁷	++++
S419	2025 and 2026	F6-F6	N-N	2-2	14-(Lig'-EWLDQEWAQVQCEVYGRGCPSEE) ₂	0	++
S420	2027 and 2028	F1-F1	N-N	1-1	17-(Ad-ESFYDWFERQLG) ₂		
S423	2029 and 2030	F1-F8	C-C	1-3	ESFYDWFERQLG K ACAWPTYWNCG	6.2*10 ⁻⁸	0
S425	2031	F1-F6	C-N	1-2	GSLDESFYDWFERQLGK-Lig'-14- Lig'-EWLDQEWAQVQCEVYGRGCPSEE	2.4*10 ⁻⁹	-
S429	2032	F6-F1	C-N	2-1	EWLDQEWAQVQCEVYGRGCPSEE-Lig'-14-Lig'-GSLDESFYDWFERQLGK	6.0*10 ⁻¹⁰	
S432	2033 and 2034	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K CEVYGRGCP S	1.8*10 ⁻⁷	+
S433	2035 and 2036	F1-F6	C-C	1-2	ESFYDWFERQLGGGG	1.1*10 ⁻⁷	+

S436	2037 and 2038	F1-F6	C-C	1-2	WLDQEWAHQ ESFYDWFERQLGGG K	5.2*10 ⁻¹⁰	+++	
S437	2039 and 2040	F1-F6	C-C	1-2	WLDQEWAHQCEVYGRGCP ESFYDWFERQLGGG K	6.9*10 ⁻¹⁰	+++	
S438	2041 and 2042	F1-F6	C-C	1-2	LDQEWAHQCEVYGRGCP ESFYDWFERQLGGG K	3.0*10 ⁻⁸	++	
S439	2043 and 2044	F1-F6	C-C	1-2	DQEWAHQCEVYGRGCP ESFYDWFERQLGGG K	4.6*10 ⁻⁸		
S440	2045 and 2046	F1-F6	C-C	1-2	QEWAHQCEVYGRGCP ESFYDWFERQLGGG K	9.9*10 ⁻⁸		
S441	2047 and 2048	F1-F6	C-C	1-2	EWAHQCEVYGRGCP ESFYDWFERQLGGG K	1.2*10 ⁻⁷		
S442	2049 and 2050	F1-F6	C-C	1-2	WAHQCEVYGRGCP ESFYDWFERQLGGG K	1.6*10 ⁻⁷		
S443	2051 and 2052	F1-F6	C-C	1-2	AWHQCEVYGRGCP ESFYDWFERQLGGG K	1.7*10 ⁻⁷		
S444	2053 and 2054	F1-F6	C-C	1-2	WVHQCEVYGRGCP ESFYDWFERQLGGG K	1.9*10 ⁻⁷		
S445	2055 and 2056	F1-F6	C-C	1-2	VHQCEVYGRGCP ESFYDWFERQLGGG K	2.3*10 ⁻⁷		
S453	2057	F1-F6	C-N	1-2	GSDDESFYDWFERQLGKK-Aid-17-Aid-KEWLDEQAWVQCEVYGRGCPSEE	5.7*10 ⁻¹⁰	-	

S454	2058 and 2059	F1-F6	C-C	1-2	GSLDESFYDWFERQLGKKK-Ald 17	3.8*10 ⁻¹⁰	+++
S455	2060	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald 17	1.1*10 ⁻⁹	+++
S456	2061 and 2062	F1-F6	N-N	1-2	Ald-GSLDESFYDWFERQLGKK 17	2.4*10 ⁻⁹	+++
					Ald-KEWLQEWAWVQCEVYGRGCPSEE		
S457	2063	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSSGGSGSLDESFYDWFERQLG	1.6*10 ⁻⁹	+++
S458	2064	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSSGGSGSLDESFYDWFERQLG	3.2*10 ⁻⁹	+++
S459	2065	F1-F6	C-N	1-2	GSLDESFYDWFERQLGGSGGSWLDQEWAWVQCEVYGRGCPSS	7.6*10 ⁻¹¹	-
S467	2066	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald-16-Ald-GSLDESFYDWFERQLGKK	6.8*10 ⁻¹⁰	+++
S468	2067	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald-19-Ald-GSLDESFYDWFERQLGKK	4.0*10 ⁻¹⁰	+++
S471	2068	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG	6.7*10 ⁻¹⁰	+++
S481	2069	F6-F1	C-N	2-1	HHHHHHKLQEWAWVQCEVYGRGCPSESFYDWFERQLG		
S482	2070	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG		
S483	2071	F6-F1	C-N	2-1	LDEWAHVQCEVYGRGCPSESFYDWFERQLG	5.2*10 ⁻⁸	0
S484	2072	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG	8.7*10 ⁻⁸	0
S485	2073	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG	1.6*10 ⁻⁷	0
S486	2074	F6-F1	C-N	2-1	LDQEWAHVQCVYGRGCPSESFYDWFERQLG	5.7*10 ⁻⁸	0
S487	2075	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG		
S488	2076	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCSSESFYDWFERQLG		
S489	2077	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG		
S490	2078	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCSSESFYDWFERQLG		
S491	2079	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSEFYDWFERQLG		
S492	2080	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQLG		
S493	2081	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEPOX-Lys(biotin)		
S494	2082	F6-F1	C-N	2-1	ADQEWAHVQCEVYGRGCPSESFYDWFERQLG	1.7*10 ⁻⁸	+
S495	2083	F6-F1	C-N	2-1	LAQEWAHVQCEVYGRGCPSESFYDWFERQL		
S496	2084	F6-F1	C-N	2-1	LDAEWAHVQCEVYGRGCPSESFYDWFERQL		
S497	2085	F6-F1	C-N	2-1	LDDQAWAWVQCEVYGRGCPSESFYDWFERQL	2.5*10 ⁻⁹	+++
S498	2086	F6-F1	C-N	2-1	LDQEAAWVQCEVYGRGCPSESFYDWFERQL	5.6*10 ⁻⁴	+
S499	2087	F6-F1	C-N	2-1	LDQEWAHVQCEVYGRGCPSESFYDWFERQL	6.2*10 ⁻¹⁰	+++

S500	2088	F6-F1	C-N	2-1	LDQEWAWAQCEVYGRGCPSESFYDWFERQL
S501	2089	F6-F1	C-N	2-1	LDQEWAWVACEVYGRGCPSESFYDWFERQL
S502	2090	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESFYDWFERQL
S503	2091	F6-F1	C-N	2-1	LDQEWAWVQCEAYGRGCPSESFYDWFERQL
S504	2092	F6-F1	C-N	2-1	LDQEWAWVQCEAVAGRGCPSSESFYDWFERQL
S505	2093	F6-F1	C-N	2-1	LDQEWAWVQCEVYARGCPSESFYDWFERQL
S506	2094	F6-F1	C-N	2-1	LDQEWAWVQCEVYAGCPSSESFYDWFERQL
S507	2095	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRACPSSESFYDWFERQL
S508	2096	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCASESFYDWFERQL
S509	2097	F6-F1	C-N	2-1	LDQEWAWVQSEVYGRGSPSESFYDWFERQL
S510	2098	F6-F1	C-N	2-1	SLEEEWAQCEVYGRGCPSSGSGSLLDESFYHWFDRLQLR
S511	2099	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSSGSGSGRVDWLQRNANFYDWFWAELG
S512	2100	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSSGGSSQAGSAFYAWFDQVLRTV
S513	2101	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSSGGSSQSDAEYSGLWALIGISDG
S515	2102	F6	-	2	LDOQEWAWVQCEVYGRGCPSPOX-Lys(Biotin)
S516	2103	F4-F1	C-N	2-1	H-ACY-CLEEwgASL-Tic-QCSGSESFYDWFERQL
S517	2104	F6-F1	C-N	2-1	SIEEEWAQIKCDWGRGCPSESFYDWFERQL
S518	2105	F6-F1	C-N	2-1	RLEEEWAWVQCEVYGRGCPSSGLDESFYDWFERQLG
S519	2106	F6-F1	C-N	2-1	SLEEEWAQCEVYGRGCPSSGLDESFYDWFERQLG
S520	2107	F6-F1	C-N	2-1	SIEEEWAQIKCDWGRGCPGGLIDESFYHWFDRLQLR
S521	2108	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSCGGGLIDESFYDWFERQL
S522	2109	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSCGGRVDWLQRNANFYDWFWAELG
S523	2110	F6-F10	C-N	2-1	WLDQEWAWVQCEVYGRGCPSPSDMAGYEWFFEEQLD
S524	2111	F6-F1	C-N	2-1	HHHHHHSLEEEWAQCEVYGRGCPGSLSDESFYDWFERQLG

S471	2068	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG
S481	2069	F6-F1	C-N	2-1	HHHHHHHKLDQEWAWVQCEVYGRGCPSESFYDWFERQLG
S482	2070	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG
S483	2071	F6-F1	C-N	2-1	LDEEWAWVQCEVYGRGCPSESFYDWFERQLG
S484	2072	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG
S485	2073	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG
S486	2074	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG

S487	2075	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRCPSESFYDWFERQLG
S488	2076	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCSESFSFYDWFERQLG
S489	2077	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRCPSESFSFYDWFERQLG
S490	2078	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCSESFSFYDWFERQLG
S491	2079	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCSESYDWFERQLG
S492	2080	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCSESYDWFERQLG
S493	2081	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGPSEE-POX-Lys(biotin)
S494	2082	F6-F1	C-N	2-1	ADQEWAWVQCEVYGRGPSESFYDWFERQLG
S495	2083	F6-F1	C-N	2-1	LAQEWAQWVQCEVYGRGPSESFYDWFERQL
S496	2084	F6-F1	C-N	2-1	LDAEWAWVQCEVYGRGPSESFYDWFERQL
S497	2085	F6-F1	C-N	2-1	LDQAWAWVQCEVYGRGPSESFYDWFERQL
S498	2086	F6-F1	C-N	2-1	LDQEAQWAWVQCEVYGRGPSESFYDWFERQL
S499	2087	F6-F1	C-N	2-1	LDQEWAAQWVQCEVYGRGPSESFYDWFERQL
S500	2088	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGPSESFYDWFERQL
S501	2089	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGPSESFYDWFERQL
S502	2090	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGPSESFYDWFERQL
S503	2091	F6-F1	C-N	2-1	LDQEWAWVQCEAYGRCGPSESFYDWFERQL
S504	2092	F6-F1	C-N	2-1	LDQEWAWVQCEVAGRGPSESFYDWFERQL
S505	2093	F6-F1	C-N	2-1	LDQEWAWVQCEVYARGCPSESFYDWFERQL
S506	2094	F6-F1	C-N	2-1	LDQEWAWVQCEVYAGCPSESFYDWFERQL
S507	2095	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRACPSESFYDWFERQL
S508	2096	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCASESFYDWFERQL
S509	2097	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGPSESFYDWFERQL
S510	2098	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGCPSGGGGSLIDESFYHWFDRQLR
S511	2099	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGGGSRDVWLQRNANFYDWFAEL
S512	2100	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGGGSSQAGSAFYAWFDQVRLTV
S513	2101	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSSQDAFYDWGLSDG
S515	2102	F6	-	2	LDQEWAWVQCEVYGRGPSPQX-Lys(Biotin)
S516	2103	F4-F1	C-N	2-1	H-Acy-CLEEWGASL-Tie-QCQSGSESFYDWFERQL
S517	2104	F6-F1	C-N	2-1	SIEEEWAQIKCDWWGRGPSESFYDWFERQL
S518	2105	F6-F1	C-N	2-1	RLEEEWAQWVQCEVYGRGPSPGSQSLDESFYDWFERQLG
S519	2106	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGPSPGSQSLDESFYDWFERQLG
S520	2107	F6-F1	C-N	2-1	SIEEEWAQIKCDWWGRGPPLIDESFYHWFDRQLR
S521	2108	F4-F1	C-N	2-1	HLCVLEELFNGASLFGYCSCGGSLIDESFYDWFERQL
S522	2109	F4-F1	C-N	2-1	HLCVLEELFNGASLFGYCSCGGRVDWLQRNANFYDWFAEL

S523	2110	F6-F10	C-N	2:1	WLDQEWAQVQCEVYGRGCPSDSDWAGYEWFEEQLD HHHHHKSLEEEWAQVECEVYGRGCPSSLDSESYDWFERQLG	$4.3 \cdot 10^{-3}$	++
S524	2111	F6-F1	C-N	2:1		$1.1 \cdot 10^{-11}$	+++++

S527		F4-F1	C-N	2:1	H-Acy-CAQEWGSEL-Tic-QCSCSESFYDWFERQL SLEEEWAQVECEVYGRGCPSESFYDWFERQL	$2.4 \cdot 10^{-3}$	
S530		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQL	$8.0 \cdot 10^{-12}$	++++++
S531		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQL	$7.5 \cdot 10^{-11}$	+++
S532		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQL	$3.7 \cdot 10^{-11}$	+++++
S533		F6-F1	C-N	2:1	LDQEWAQVQCEVYGRGCPSESFYDWFERQL	$6.7 \cdot 10^{-11}$	+++++
S534		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQL	$1.0 \cdot 10^{-11}$	+++++
S535		F6-F1	C-N	2:1	QLDEEWAGVQCEVYGRGCSLDSESYDWFERQLG		
S536		F6-F1	C-N	2:1	LEEWAQVECEVYGRGCPSESFYDWFERQL	$8.3 \cdot 10^{-11}$	+++++
S537		F6-F1	C-N	2:1	SLEHEWAQVECEVYGRGCPSSLDSESYDWFERQLG	$4.4 \cdot 10^{-11}$	+++++
S538		F6-F1	C-N	2:1	SLEQEWAWQVECEVYGRGCPSSLDSESYDWFERQLG	$3.8 \cdot 10^{-11}$	++++
S539		F6-F1	C-N	2:1	SLELEWAQVECEVYGRGCPSSLDSESYDWFERQLG	$9.8 \cdot 10^{-11}$	+++++
S540		F6-F1	C-N	2:1	SLEEEWAQVKCEVYGRGCPSSLDSESYDWFERQLG	$1.3 \cdot 10^{-11}$	+++++
S541		F6-F1	C-N	2:1	SLEEEWAQVECEVWGRGCPSSLDSESYDWFERQLG	$7.8 \cdot 10^{-12}$	++++++
S542		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSSLDSESYHWFERQLG GSLDSESYDWFERQLGGSGGSWILDEEWAQVQCEVYGRGCPSS	$2.7 \cdot 10^{-11}$	++++++
S543		F1-F6	C-N	1:2	ESFYDWFERQLGWLDQEWAQVQCEVYGRGCPSS	$1.9 \cdot 10^{-11}$	—
S544		F1-F6	C-N	1:2	ESFYDWFERQLGWLDQEWAQVQCEVYGRGCPSS		
S545		F1-F6	C-N	1:2	ESFYDWFERQLGWLDQEWAQVQCEVYGRGCPSS		
S546		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSSLDSESYDWFERQ-Bpa-GK(Biotin)	$2.6 \cdot 10^{-3}$	
S547		F6		2	SLEEEWAQVECEVYGRGCPSS	$4.9 \cdot 10^{-3}$	-
S548		F6		2	SLEEEWAQVECEVWGRGCPSS	$4.1 \cdot 10^{-3}$	-
S549		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSSLDSESYDWFERQLG Ac-GSLDESFYDWFERQLG-POX-K	$1.3 \cdot 10^{-11}$	++++++
S550		F1		1		$4.0 \cdot 10^{-3}$	
S551		F6-F1	C-N	2:1	SLEEEWAQVEAEVYGRGAPGSSLDSESYDWFERQLG	$7.2 \cdot 10^{-11}$	
S552		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSSLDSESYDWFERQLGKHHHHH	$7.3 \cdot 10^{-12}$	
S553		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGPPGLLDSESYHWFDRLQR	$6.4 \cdot 10^{-12}$	++++++
S554		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQLG	$5.7 \cdot 10^{-11}$	++++
S555		F6-F1	C-N	2:1	SLEEEWAQVECEVYGRGCPSESFYDWFERQLG	$3.2 \cdot 10^{-11}$	++++
S556		F6-F1	C-N	2:1	SLEEEWAQIKCDWWGRGCSESFYDWFERQL		

S557	F6-F1	CN	2-1	SLEEEWAQIECEVYGRGCPSESFYDWFERQL	2.0*10^-11	
S558	F6-F1	CN	2-1	SLEEEWAQIECEVWGRGCPSESFYDWFERQL	1.9*10^-11	++++++
S559	F6-F1	CN	2-1	SLEEEWAQIECEVWGRGCPSESFYDWFERQL	2.1*10^-11	++++++
S560	F6-F1	CN	2-1	SLEEEWAQIECEVWGRGCPGSILDESFYDWFERQL	1.4*10^-11	++++++
S561	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGCPGSILDESFYDWFERQL	1.8*10^-11	++++++
S562	F6-F1	C-N	2-1	SIEEEWAQIECEVWGRGCSLIDESFYDWFERQL	1.8*10^-11	+++++
S563	F6-F1	C-N	2-1	SIEEEWAQIKCDWGRCSSESFYDWFERQL	1.4*10^-11	+++++
S564	F6-F1	C-N	2-1	SIEEEWAQIQCEVWGRCSSESFYDWFERQL	1.3*10^-11	++++++
S565	F6-F1	C-N	2-1	SIEEEWAQIQCEVWGRGPSESFYDWFERQL		
S566	F6-F1	C-N	2-1	SIEEEWAQVECEVWGRGPSESFYDWFERQL		
S567	F6-F1	C-N	2-1	SIEEEWAQIQCEVWGRGPSESFYDWFERQL		
S568	F6-F1	C-N	2-1	AcSIEEEWAQIKCDWGRGPSESFYDWFERQL	4.3*10^-12	++++++
S569	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	1.5*10^-10	+++
S570	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	7.3*10^-10	+++
S571	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	1.6*10^-9	
S572	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	4.8*10^-9	
S573	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	3.6*10^-11	+++
S574	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL	9.2*10^-12	++++
S575	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL		
S576	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL		
S577	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL		
S578	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL		
S579	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGPSESFYDWFERQL		
S580	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGC-Bsum-ESFYDWFERQL	1.2*10^-11	++++
S581	F6-F1	C-N	2-1	SLEEEWAQIECEVWGR-Bsum-ESFYDWFERQL	1.2*10^-11	+++
S582	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGCPKGFYGWFRRRRG	2.5*10^-9	
S583	F6-F1	C-N	2-1	ELEEEWAQIECEVWGRGCPKGFYGWFRRRRG		
S584	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGCPSESFYDWFERQL		
S585	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGCPSESFYDWFERQL		
S586	F6-F1	C-N	2-1	SLEEEWAQIECEVWGRGCPSESFYDWFERQL		
S587	F6-F1	C-N	2-1	ELEEEWAQIECEVWGRGCPKGFYGWFRRRRG		
S588	F6-F1	C-N	2-1	LEEWAQVECEV-lodoTy-GRGCGSGLDESFYDWFERQL	1.8*10^-10	++++
S589	F6-F1	C-N	2-1	LEEWAQVECEVYGRGCGSGLDESFYDWFERQL		
S590	F6-F1	C-N	2-1	LEEWAQIECEV-lodoTy-GRGCGSGLDESFYDWFERQL	5.8*10^-11	++++
S591	F6-F1	C-N	2-1	LEEWAQIECEVWGRGCGSGLDESFYDWFERQL	1.3*10^-10	++++
S592	F6	2		SLEEEWAQIECEVWGRGCPSY	1.7*10^-9	

S593	F6	C-N	2	SIEEEWAQIKCDWGRGCPSY	2.2*10 ⁻⁹	
S594	F6-F1	C-N	2-1	SLEEEWAQIQLCEVWGRCWHHHSFYDWFERQL	7.1*10 ⁻¹¹	+++++
S595	F6-F1	C-N	2-1	LEEWEAQIQLREWWHSPASESFYDWFERQL	6.2*10 ⁻¹⁰	++++
S596	F6-F1	C-N	2-1	SLEEEWAQIQHELYGAESSESFYDWFERQL	4.5*10 ⁻¹¹	++++
S597	F6-F1	C-N	2-1	AC-SLEEEWAQIECEVYGRGCPSESFYDWFERQL	8.5*10 ⁻¹²	++++++
S600	F6-F1	C-N	2-1	AC-SIEEEWAQIKCDWGRGSESFYDWFERQL	7.6*10 ⁻¹²	
S601	F6-F1	C-N	2-1	SLEEEWAQIQEDLYGANHNESFYDWFERQL	1.8*10 ⁻¹⁰	
S602	F6-F1	C-N	2-1	SLEEEWAQIQAEVYGNPNSESFYDWFERQL	3.1*10 ⁻¹¹	
S603	F6-F1	C-N	2-1	AC-SLEEEWAQIQEDLYGANHNESFYDWFERQL	1.5*10 ⁻¹¹	
S604	F6-F1	C-N	2-1	SLEEEWAQIQCEVWGRGCWRRHIFYDWFERQL		
S605	F6-F1	C-N	2-1	SLEEEWAQIQHELYWPVEKGESFYDWFERQL	9.4*10 ⁻¹¹	++++
S606	F6-F1	C-N	2-1	SLEEEWAQIQOCEVWGRGCPSESFYDWFERQL	4.0*10 ⁻¹²	++++++
S607	F6-F1	C-N	2-1	SLEEEWAQIQCKLYGRNCKESFYDWFERQL		
S608	F6-F1	C-N	2-1	SLEEEWAQIQCKWGRKCKESFYDWFERQL		
S609	F6-F1	C-N	2-1	SLEEEWAQIQCKLYGRNCKSESFYDWFERQL		
S610	F6-F1	C-N	2-1	SLEEEWAQIQCKLWGKNCKESFYDWFERQL		
S611	F6-F1	C-N	2-1	SLEEEWAQIQCEVWGRGCPSESFYDWFERQLPK		
S612	F6-F1	C-N	2-1	HQLEEEWQAIQCELWGRGCPSESFYDWFERQL		
S613	F6-F1	C-N	2-1	HLEEEWSEIQCELWGRGCPSESFYDWFERQL		
S614	F6-F1	C-N	2-1	SLEEEWAQIQCEVYGRGCPSEDFYDWFEAQLHA		
S615	F6-F1	C-N	2-1	AC-SLEEEWAQIECEVYGRGCPSEDFYDWFEAQLHA		
S616	F6-F1	C-N	2-1	HQLEEEWQAIQCELWGRGCPSEDFYDWFEAQLHA		
S617	F6-F1	C-N	2-1	HLEEEWSEIQCELWGRGCPSEDFYDWFEAQLHA		
S618	F6-F1	C-N	2-1	HELEEEWKRIECELWGRGCPSEDFYDWFEAQLHA		
S619	F6-F1	C-N	2-1	AC-HQLEEEWQAIQCELWGRGCPSEDFYDWFEAQLHA		
S620	F6-F1	C-N	2-1	AC-HLEEEWKRIECELWGRGCPSEDFYDWFEAQLHA		
S621	F6-F1	C-N	2-1	AC-HLEEEWKRIECELWGRGCPSEDFYDWFEAQLHA		
S622	F6-F1	C-N	2-1	SLEEEWAQIQCEVWGRGCPSESFYDWFERQLG		
S623	F6-F1	C-N	2-1	AC-SLEEEWAQIECEWGRGCPSESFYDWFERQLG		
S624	F6-F1	C-N	2-1	SLEEEWAQVECEV-(3-iodo-Tyr)-GRGCPGSSLDESFYDWFERQLG-NH ₂		
S625	E8		1	KVRGFQQGTWPGYEWLRNAAKK		
S626	F6-E8	C-N	2-1	SLEEEWAQIQCEVYGRGCPSVRGFQQGTWPGYEWLNAAA		
S627	F6-F1	C-N	2-1	AC-SLEEEWAQIQHELW/PVEKGESFYDWFERQL		
S628	F6-F1	C-N	2-1	AC-HGLEEEWAQIQHELW/PVEKGESFYDWFERQL		
S629	F6-F1	C-N	2-1	HLEEEWRQIQCELWGRGCPSESFYDWFERQL		
S630	F6-F1	C-N	2-1	AC-HLEEEWRQIQCELWGRGCPSESFYDWFERQL		

S631		F6-F1	C-N	2-1	HPILEEEWSQIQCELWGRGCPSESFYDWFERQL
S632		F6-F1	C-N	2-1	Ac-HPLEEEWSQIQCELWGRGCPSESFYDWFEAQHLA
S633		F6-F1	C-N	2-1	HGLEEEWAQIQCEWGRGCPSESFYDWFEAQHLA
S634		F6-F1	C-N	2-1	Ac-SLEEWAQIQCEWGRGCPSESFYDWFEAQHLA
S635		F6-F1	C-N	2-1	Ac-SLEEWAQIECEVYGRGCPSEDFYDWFEQLHN
S636		F6-F1	C-N	2-1	Ac-SLEEWAQICEWGRGCPSESFYDWFERQL
S637		F6-F2	C-N	2-1	Ac-SLEEWAQIECEVYGRGCPSDGFYNAIELLS
S638		F6-F1	C-N	2-1	Ac-HGLEEEWAQIQCEWGRGCCORPEPFYDWFEAQHLA
S639		F6-F1	C-N	2-1	Ac-HGLEEEWAQIQCEWGRGCPSESFYDWFEAQHLA
S640		F6		2	SLEEEWAQIQHELWPVEAGESY
S641		F6-F1	C-N	2-1	Ac-SLEEEWAQIQAEWGRGAPSESFYDWFEAQHLA
S642		F6-F1	C-N	2-1	Ac-SLEEWAQIQCEWGRGCQRPEPFYDWFERQL
S643		F6-F1	C-N	2-1	Ac-SLEEEWAQIQCELWGRGCPSESFYDWFERQL
S644		F6-F1	C-N	2-1	SLEEEWAQHQHEDVYHPPAESFYDWFERQL
S645		F6-F1	C-N	2-1	Ac-HGLEEEWAQHEEDVYHPPAESFYDWFEAQHLA
S646		F6-F1	C-N	2-1	Ac-SLEEEWAQIQCEWGRGCHINHLPFYDWFERQL
S647		F6-F1	C-N	2-1	Ac-SLEEEWAQIQCEWGRGCPSEPFYDWFAHDNGD
S648		F6-F1	C-N	2-1	Ac-SLEEEWAQIQCEWGRGCPSEAFYDWFAEQLDD

7, 9, 12, 13, 14, 17, 19, 20, 21, 22, 23, and 24 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist. Peptides listed on 3 lines consist of two different peptides, linked N-N or C-C, either by chemical linkage or by being synthesized on the two branches of an amino acid with two amino groups such as, e.g., lysine. Acy = 1-amino-1-cyclohexanecarboxylic acid; Cha = cyclohexylalanine; Alb = 2-aminoisobutyric acid; Hyp = Hydroxyproline; Amino acids which are not capitalized are D-amino acids; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH₂-O-NH₂) on the side chain amino group; Lig' = lysine with a 2-aminohydroxyacetyl group (CO-CH₂-O-NH₂) on the side chain amino group; Ald = an aldehyde group obtained by periodate oxidation of a serine, either N-terminal or attached to the side chain amino group of lysine.

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Results further indicated that S175-S175 dimer peptides (Site 1-Site 1) were less agonistic than S175 monomer peptides (++ vs. +++). S175-S175 dimer peptides having a C-N linkage were less agonistic or equally agonistic as compared to S175-S175 dimer peptides having C-C or N-N linkages. F8-F8 dimer peptides, like the parent monomer, showed no agonist activity.

Table 7 further indicates that, relative to peptide S519, a potent insulin mimetic, the alterations that are most influential in increasing receptor affinity and potency are: acetylation of the N-terminal amino group; 10 replacing V at position 9 with I; replacing E at position 10 with Q; replacing Y at position 14 with W; and deleting the sequence GSLD at positions 21 to 24.

Example 5: Substrate Phosphorylation Assay (HIR Kinase)

WGA (wheat germ agglutinin)-purified recombinant human insulin receptor was mixed with either insulin or peptide in varying concentrations in substrate phosphorylation buffer (50 mM HEPES (pH 8.0), 3 mM MnCl₂, 10 mM MgCl₂, 0.05 % Triton X-100, 0.1 % BSA, 12.5 μM ATP). A synthetic biotinylated substrate peptide (Biotin-KSRGDYMTMQIG) was added to a final concentration of 2 μg/ml. Following a 1 hr incubation at RT, the reactions were stopped by the addition of 50 mM EDTA. The reactions were transferred to Streptavidin coated 96-well microtiter plates (NUNC, Cat. No. 236001) and incubated for 1 hr at RT. The plates were washed 3 times with TBS (10 mM Tris (pH 8.0), 150 mM NaCl).

Subsequently, a 2000-fold dilution of horseradish peroxidase (HRPO) conjugated phosphotyrosine antibody (Transduction Laboratories, Cat. No. E120H) in TBS was added. The plates were incubated for 30 min and washed 3 times with TBS. TMB (3,3',5,5'-tetramethylbenzidine; Kem-En-Tec, Copenhagen, Denmark) was added. One substrate from Kem-En-Tec was added. After 10-15 min, the reaction was stopped by the addition of 1% acetic acid. The absorbance, representing the extent of substrate

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phosphorylation, was measured in a spectrophotometer at a wavelength of 450 nM.

The results indicated that the potency of the Site 1-Site 2 dimer, peptide 539, was 0.1 to 1% of that of insulin in all assays tested (Table 8), 5 and the dose-response curves (Figures 17A-17B) had a shape similar to that of insulin dose-response curves, suggesting an insulin-like action mechanism. In addition, Site 1-Site 2 dimer peptides 537 and 538 were also active as specific insulin receptor antagonists (Table 8; Figures 16A-16C). Notably, Site 2-Site 1 dimer peptide 539 was more active in the kinase 10 assay than Site 1-Site 1 homodimer peptides 521 and 535 (Figures 19A-19B), despite lower FFC potency (Figures 14A-14C; Figures 17A-17B). Similar results are shown in Figures 20A-B and Figures 21A-B. This data suggested that homodimer and heterodimer peptides used different mechanisms of action.

15

TABLE 8

Pep.	Mon./Link.	Sequence	SEQ ID NO:	Form	Site IR	HIR K _d (nM)	HIGF-1R K _d (nM)	FFC Pot. (nM)	Kinase Pot. (nM)
HI				na	na				
HIGF-1R				na	na				
521	RP9-6aa-RP9	MADYKDDDKGSLDESFYDWFER QLGKKGGSGGSGLDESFYDWFE RQLGKAAA(ETAG)PG	2112	1-1	1-1	25	-	A 3	1400
535	RP9-12aa-RP9	MADYKDDDKGSLDESFYDWFER QLGKKGGSGGSGLDES FYDWFERQLGKAAA(ETAG)PG	2113	1-1	1-1	15	-	A 2	1000
537	RP9-6aa-D8	MADYKDDDKGSLDESFYDWFER QLGKKGGSGSWLDQEWAQVQC EVYGRGCPAAA(ETAG)PG	2114	1-6	1-2	0.092	980	N 10	Inactive
538	RP9-12aa-D8	MADYKDDDKGSLDESFYDWFER QLGKKGGSGSGSGLDES WAWVQCEVYGRGCPAAA(ETAG) PG	2115	1-6	1-2	0.080	710	N 10	Inactive
539	D8-6aa-RP9	MADYKDDDKWLDQEWAQVCE VYGRGCPGGSGSGSGLDESFYD WFERQLGKAAA(ETAG)PG	2116	6-1	2-1	0.530	1500	A 10	110

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A = agonist; N = antagonist; na = not applicable; Form. = formula; Mon. = constituent monomers; Link. = linker; Pot. = potency; H1 and HIGF-1R are controls; All with tags at both ends; All dimers are linked C-N; Linker sequences are underlined.

Example 6: IR Autophosphorylation Assays

5 IR activation was assayed by detecting autophosphorylation of an insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993, *Science* **261**:1591-1594; clone 969). The IR transfected 32D cells were seeded at 5×10^6 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium
10 (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10
15 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 hr incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

30 Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression

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System) was incubated with 25 nM insulin, or 10 or 50 μ M peptide in 50 μ l autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM MnCl₂, 50 μ M sodium orthovanadate) containing 10 μ M ATP for 45 min at 22°C. The reaction was stopped by adding 50 μ l of gel 5 loading buffer containing β -mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 10 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 7: Fluorescence-Based HIR Binding Assays

15 **A. Time-Resolved Fluorescence Resonance Energy Transfer Assays**

Time-resolved fluorescence resonance energy transfer assays (TR-FRET) were used for peptide competition studies. In one set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated RP-9 monomer peptide (b-RP9) for binding to HIR- 20 immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996). The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μ l. Final incubation conditions were in 22 nM b-RP9, 1 nM SA-APC (streptavidin-allophycocyanin), 1 nM Eu³⁺-sIR-Fc (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 25 0.0027 M KCl, and 0.1 % BSA (Cohn Fraction V). After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as percent of specific binding.

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Results are shown in Figures 22A-22B. Figure 21A shows b-RP9 competition data. For these figures, the Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al., 1999, *J. Biomol. Screen.* 4:67-73), and the signal-to-background (S/B) ratio was ~4-5. In Figure 22A, each data point 5 represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data according to the following formula: $y = \min + (\max - \min)/(1 + 10^{((\log IC_{50}) - x) * \text{Hillslope}})$. This was used to determine IC_{50} values.

In another set of assays, monomer and dimer peptides were tested 10 for the ability to compete with biotinylated-S175 (b-S175) or b-RP9 for binding to sIR-Fc. The TR-FRET assays were performed in a 384-well white microplate with a final volume of 30 μl . Final incubation conditions were in 33 nM b-S175 or 22 nM b-RP9, 1 nM SA-APC, 1 nM Eu³⁺-sIR-Fc, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1 % BSA. 15 After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader. Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding.

Results are shown in Figures 23A-23B. For these figures, each data 20 point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC_{50} values. Figure 23A shows b-S175 competition data; Figure 23B shows b-RP9 competition data.

B. Fluorescence Polarization Assays

Fluorescence polarization assays (FP) were used for peptide 25 competition studies. In one set of assays monomer and dimer peptides were tested for the ability to compete with fluorescein-RP-9 (FITC-RP9) for binding to soluble HIR ectodomain (sIR; Kristensen et al., 1998, *J. Biol. Chem.* 273:17780-17786). The assays were performed in a 384-well black 30 microplate (NUNC) with a final volume of 30 μl . Final incubation conditions

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were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05 % BGG (bovine gamma globulin), 0.005 % Tween-20®. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.).

5 Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls, and then expressed as percent of specific binding. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. In Figures 24-27, each data point represents the average of two replicate wells. The lines represent the best fit to a four-
10 parameter non-linear regression analysis of the data, which was used to determine IC₅₀ values. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. Results are shown in Figures 24A-24B.

In another set of assays, monomer and dimer peptides were tested for the ability to compete with FITC-RP9 for binding to soluble human insulin
15 mini-receptor (mIR; Kristensen *et al.*, 1999, *J. Biol. Chem.* **274**:37351-37356). The FP assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were 2 nM FITC-RP9, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.001 % BGG, 0.005 % Tween-20®. After 16-24 hr of incubation at RT, the
20 fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 25A-25B.

Monomers and dimer peptides were also tested for the ability to
25 compete with fluorescein-insulin (FITC-Insulin) for binding to sIR. The FP assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were in 2 nM FITC-Insulin, 20 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05 % BGG, 0.005 % Tween-20®. After 16-24 hr of incubation at RT, the fluorescence
30 signal at 520 nm was read on an Analyst™ AD plate reader. Primary data

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were background corrected using 20 nM sIR without FITC-Insulin addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 26A-26B.

In other assays, peptide monomers and dimer peptides were tested
5 for the ability to compete with FITC-Insulin for binding to mIR. The FP assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were 2 nM FITC-Insulin, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05 % BGG (bovine gamma globulin), 0.005 % Tween-20®. After 16-24 hr of incubation
10 at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % Specific Binding. Results are shown in Figures 27A-27B.

C. Summary

15 Table 9, below, summarizes the binding data calculated from competition assays using the IR constructs, sIR-Fc, sIR, and mIR, in TR-FRET and FP formats. The data in Table 9 indicate that most dimer peptides (e.g., S291 and S375 or S337), showed greater agonist activity than the corresponding monomer peptides (e.g., H2C or RP9, respectively) in the FFC assay. It was previously demonstrated that an inequality between monomer peptides and insulin was exhibited in competition assays where the assay reporter was a monomer peptide (i.e., RP9 or S175). This inequality was also demonstrated by dimer peptides as seen in Table 9. Table 9 further shows that Group 6 monomer peptides such as E8 (D120)
20 were able to compete with FITC-RP9 or b-RP9 peptides for binding to sIR-Fc, but did not compete peptide ligands, such as FITC-RP9 for binding to mIR. Experiments using different IR constructs thereby allowed differentiation of Site I peptides based on sequence motifs (i.e., Group 6 (Formula 10) vs. Group 1 (Formula 1; A6)).
25

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TABLE 9

Monomer SEQ ID NO: or Dimer	Link- age	TARGET \Rightarrow		sIR-Fc		sIR-Fc		sIR		mIR		HIR	
		Label \Rightarrow		b-S175	b-RP9	FRET	FRET	FITC-RP9	FITC-RP9	FP	FP	FITC-RP9	Insulin
		(nM)	(nM)			(nM)	(nM)	(nM)	(nM)			(nM)	(nM)
H2C	2117	FHENFYDWVFRVSKK	410	-0.82	1626	-1.03	50	-0.27	37	-0.49	770	-0.89	700
S291 and N-N	N-N (Lg-GGG+H2C) ₂ -9	81	-0.96	250	-0.69			12	-0.35	668	-0.38	1200	+++
RP9	1558	GSLDESFYDWFERQLGKK	6	-0.45	42	-0.69	10	-0.41	0.03	-0.29	49	-0.53	44
S375	1994 C-N (RP9-Lig)-14-(RP9-Lig)	7	-0.80	86	-0.67			0.2	-0.22	91	-0.80	200	+++
S337 and C-C	(RP9-Lig) ₂ -23	0.2	-0.36	14	-0.57	1	-0.37	0.2	-0.28	111	-0.70	11	++++
S391	2008 truncated-(GSLDE)RP9(-KK)	59	-0.59	610	-0.56			119	-0.49	284	-0.77	1500	NN
S390	1794 truncated-(GSLD)RP9(-KK)	27	-0.49	127	-0.49			19	-0.64	94	-0.94	620	+
S414 and C-C	(truncated-(GSLD)RP9(-KK)) _x -14	92	-0.62	164	-0.73			0.2	-0.25	151	-0.69	NN	NN
S175	1560 GRDWLQRNNANFYDWFAELG	22	-0.58	64	-0.74	10	-0.56	1	-0.36	167	-1.72	230	+++
S380 and C-C	(EE-short-S175-Lig) _x -9	10	-0.55	23	-0.64			0.5	-0.29	27	-0.49	510	++
E8 (D120)	2118 GGTWWPGYEWLRNA	755	-0.74			207	-0.49		>100000			2200	-
Insulin		59	-0.37	63	-0.46	>100000	-0.25	1250	-	172	-0.78	0.04	++++

FRET = Time-Resolved Fluorescence Energy Transfer Assay; FP = Fluorescence Polarization Assay; RRA = Radio-Receptor Assay; FFC = Free Fat Cell Assay; N-N = N-terminal linkage; C-C = C-terminal linkage; All are site 1 (formula 1) monomers or site 1-site 1 (formula 1-formula 1) dimers;

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Based on the functional studies outlined above, the following peptide dimers were designed.

SEQ ID NO:	Monom/J Linkers	Sequence
2119	F8-6aa-RP9	HLCVLEELFWGASLFGYCSGGGGSGGSGLDESFYDWFERQL
2120	F8-12aa-RP9	HLCVLEELFWGASLFGYCSGGGGSGGSGGSGSGLDESFYDWFERQL
2121	D8-6aa-S175	WLDQEWAQVQCEVYGRGCPGGSGGGSGSGRVDWLQRNANFYDWFVAELG
2122	D8-12aa-S175	WLDQEWAQVQCEVYGRGCPGGSGGGSGSGRVDWLQRNANFYDWFVAELG
2123	F8-6aa-S175	HLCVLEELFWGASLFGYCSGGGGSGGSGRVDWLQRNANFYDWFVAELG
2124	F8-12aa-S175	HLCVLEELFWGASLFGYCSGGGGSGGSGGSGRVDWLQRNANFYDWFVAELG
2125	D8-6aa-RP15	HLCVLEELFWGASLFGYCSGGGGSGSSQAGSAFYAWFDQVLRTV
2126	D8-6aa-RP6	HLCVLEELFWGASLFGYCSGGGGSGGSTFYSCASLLTGTQPQPNRGWPWERCR
2127	D8-6aa-RP17	HLCVLEELFWGASLFGYCSGGGGSGGSQDAFYSGLWALIGLSDG
2128	D8-6aa-Grp 6	HLCVLEELFWGASLFGYCSGGGGSGGSDDWAGYEWFEEQLD

- 5 Linker sequences are underlined and in bold; Monomer sequences are shown below; All dimers are linked C-N.

SEQ ID NO:	Monomer	Formula	Site	Sequence
1576	F8	4	2	HLCVLEELFWGASLFGYCSG
1558	RP9	1	1	GSLDESFYDWFERQL
2129	D8	6	2	WLDQEWAQVQCEVYGRGCP
1560	S175	1	1	GRVDWLQRNANFYDWFVAELG
2130	RP15	1	1	SQAGSAFYAWFDQVLRTV
1635	Rp6	2	1	TFYSCASLLTGTQPQPNRGWPWERCR
2131	RP17	1	1	QSDAFYSGLWALIGLSDG
1595	Group 6	10	1	DSDWAGYEWFEEQLD

Example 8: Peptide Fusions To The Maltose Binding Protein

10 A. Cloning

The transfer of interesting peptide sequences from phage display to maltose binding protein (MBP) fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage

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display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and
5 the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 28 shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a
10 shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *Eco*RI and *Hind*III and then treating the
15 fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which
20 anneal to sequences encoding the shortened FLAG® or E-Tag epitopes and also contain the required restriction enzyme sites *Eco*RI and *Hind*III. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick
25 spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain ER2508 (RR1 *lon*:*mini*Tn10(*Tet*’) (*malB*) (*argF-lac*)U169 Pro⁺ *zjc*::Tn5(*Kan*’) *fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C)
30 2xYT medium containing 2 % glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated

onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

5 *E. coli* ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD₆₀₀ of 0.1. When the cultures reached an OD₆₀₀ of 0.6, expression was induced by the addition of IPTG to
10 a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

15 **C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins**

15 *E. coli* ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD₆₀₀ of 0.1 and grown
20 at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l): glucose (3.00); (NH₄)₂SO₄ 5.00; MgSO₄ • 7H₂O (0.25); KH₂PO₄ (3.00); citric acid (3.00); peptone (10.00); and yeast extract (5.00); pH 6.8.

25 The culture was grown at 700 rpm, 37°C until the glucose from the medium was consumed (OD₆₀₀ = ~6.0 – 7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50 % glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE

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followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

The cell pellets were disrupted mechanically by sonication or
5 chemically by treatment with the mild detergent Triton X-100. After removal
of cell debris by centrifugation, the soluble proteins were prepared for
chromatographic purification by dilution or dialysis into the appropriate
starting buffer. The MBP fusions were initially purified either by amylose
affinity chromatography or by anion exchange chromatography. Final
10 purification was performed using anti-E-Tag antibody affinity columns
(Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-
buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer
(100 mM glycine, pH 3.0). The purified proteins were analyzed for purity
and integrity by SDS-PAGE and Western blot analysis according to standard
15 protocols.

For MBP fusions, IR agonist activity was observed for the Site 1-Site
1 dimer peptides shown in Table 10, below. Additional binding data for the
MBP fusions are shown in Table 11, also below.

TABLE 10

Fus.	Monomer/ Linker	Sequence	SEQ ID NO:	Form.	Act.	Site IR	Fus. Conc.	MW (kDa)	Kd(HIR)
426	D8	MBP...NNNNLIGEGRISEFIEGR AQPAMA WLDQEWAAVQCEVYGRGCPSSAAA (ETAG)AA	2132	6	N	2	0.76	52.2	1.4 x 10 ⁻⁶
429	D8-6aa-D8	MBP...NNNNLIGEGRISEFIEGR AQPAMA WLDQEWAAVQCEVYGRGCPSSGGGS KWLDEQEWAAVQCEVYGRGCPSSAAA(ETAG)AA	2133	6-6	NN	2-2	3.2	55.3	1.3 x 10 ⁻⁶
430	H2C-4aa-RB6	MBP...NNNNLIGEGRISEFIEGR DYKDDDKFHENFYDWFWVRQVS GSSLDALDRLM RYFEERPSLETAG	2134	1-6	A-	1-1	0.17	54.5	2.1 x 10 ⁻⁶
431	H2C-6aa-F8	MBP...NNNNLIGEGRISEFIEGR DYKDDDKFHENFYDWFWVRQVS GSSGGSHLCVLEE LFWGASLFGYCSAAA(ETAG)AA	2135	1-4	A-N	1-2	3.3	54.8	4.7 x 10 ⁻⁸
432	H2C-12aa-F8	MBP...NNNNLIGEGRISEFIEGR DYKDDDKFHENFYDWFWVRQVS GSSGGSGGGSS HLCVLEELFWGASLFGYCSAAA(ETAG)AA	2136	1-4	A-N	1-2	2.9	55.5	3.5 x 10 ⁻⁸
433	H2C-9aa-F8	MBP...NNNNLIGEGRISEFIEGR DYKDDDKFHENFYDWFWVRQVS GSSGGSGGHLC VLEELFWGASLFGYCSAAA(ETAG)AA	2137	1-4	A-N	1-2	2.8	55.2	2.1 x 10 ⁻⁸
434	G3-12aa-G3	MBP...NNNNLIGEGRISEFIEGR AQPAMARGGGTFYEW FESALRKHGAGGGGGSG GSGGSRGGTFYEW FESALRKHGAGAAA(ETAG)AA	2138	1-1	NN	1-1	0.01	56	3.2 x 10 ⁻⁶
436	H2C-9aa-H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVS GSSGGSGSFHEN FYDWFVRQVSAAA(ETAG)AA	2139	1-1	A	1-1	1.1	54.2	4.1 x 10 ⁻⁷
437	H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVSAAA(ETAG)AA MBP...NNNNLIGEGRISEFIEGR AQPAMARGGGTFYEW FESTLRKHGAGGGGGSR	2140	1	NN	1	0.3	51.5	8.3 x 10 ⁻⁶
427	G3-6aa-G3	MBP...NNNNLIGEGRISEFIEGR AQPAMARGGGTFYEW FESTLRKHGAGGGGGSR GGGTTFYEW FESALRKHGAGAAA(ETAG)AA	2141	1-1	NN	1-1	0.02	55.3	3.3 x 10 ⁻⁶
435	H2C-3aa-H2C- 3aa-H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVS GSSGGSFHEN RQVSGSSFHENFYDWFWVRQVS GSSGGSFHENFYDWFWVRQVS GSSGGSFHEN RQVSGSSFHENFYDWFWVRQVS GSSGGSFHENFYDWFWVRQVS GSSGGSFHEN	2142	1-1-1	AA-A	1-1-1	2.1	55.5	2.0 x 10 ⁻⁶
439	H2C-6aa-H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVS GSSGGSFHEN FYDWFVRQVS(ETAG)AA	2143	1-1	A-A	1-1	1.4	53.9	5.5 x 10 ⁻⁷
449	H2C-12aa-H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVS GSSGGSGGGSA QPAMAFHENFYDWFWVRQVSAAA(ETAG)AA	2144	1-1		1-1	1.5	51.8	6.2 x 10 ⁻⁷
452	G3	MBP...NNNNLIGEGRISEFIEGR AQPAMARGGGTFYEW FESALRKHGAGAA A(ETAG)AA	2145	1		1	0.15	48.8	7.8 x 10 ⁻⁷
463	H2C-3aa-H2C	MBP...NNNNLIGEGRISEFIEGR AQPAMAFHENFYDWFWVRQVS GSSGFHENFYDWFW RQVSSAAA(ETAG)AA	2146	1-1	A-A	1-1	1.8	50.1	9.6 x 10 ⁻⁷

464	LF-H2C	MBP ...NNNNLGIERGRISEFIEGRDYKDDDK FHENFYDWFWVRQVSAA(ETAG)AA	2147	1	1	0.045	48.4	3.9×10^{-8}
446	LF-F8	MBP ...NNNNLGIERGRISEFIEGRDYKDDDKHLCVLEELFWGASLFGYCSGAAA(ETA G)AA	2148	1	2	1.9	49.1	7.7×10^{-7}
459	SF-RB6	MBP ...NNNNLGIERGRISEFGSADYKDLDRIMRYFEERPSLAA(ETAG)AA	2149	3	1	0.069	48.1	7.7×10^{-8}
MB P*	lacZ	**		na	5.1	50	> 1 x 10 ⁻⁵	

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ); **MBP-lacZ fusion protein was derived from the plasmid pMal-c2 as purchased form NEB. Fus. = fusion; Act. = activity; Conc. = concentration; N = Antagonist; A = Agonist; LF = Long FLAG® epitope (DYKDDDDK; SEQ ID NO:1777); SF = Short FLAG® epitope (DYKD; SEQ ID NO:1545); na = not applicable; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

TABLE 11

Fusion	Monomer/ Linker	Sequence	SEQ ID NO:	Form. IR	Site IR	High conc. tested (μM)	Kd (H/R) μM
431-	H2C-6aa-F8	MBP ...NNNNLGIERGRISEFIEGRDYKDDDKFHENFYDWFWVRQVSGGGSGSHLCVLEELFWGASLFGYCS GAAA(ETAG)AA	2150	1-6	1-2	0.2	0.033
431+	H2C-6aa-F8	DYKDDDKFHENFYDWFWVRQVSGGGSGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2151	1-6	1-2	0.2	0.0074
432-	H2C-12aa-F8	MBP ...NNNNLGIERGRISEFIEGRDYKDDDKFHENFYDWFWVRQVSGGGSGGGSGSHLCVLEELFWGAS SLFGYCSGAAA(ETAG)AA	2152	1-6	1-2	0.2	0.02
432+	H2C-12aa-F8	DYKDDDKFHENFYDWFWVRQVSGGGSGGGSGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2153	1-6	1-2	0.2	0.0038
433-	H2C-9aa-F8	MBP ...NNNNLGIERGRISEFIEGRDYKDDDKFHENFYDWFWVRQVSGGGSGGGSGSHLCVLEELFWGASLFG YCSGAAA(ETAG)AA	2154	1-6	1-2	0.2	0.03
433+	H2C-9aa-F8	DYKDDDK FHENFYDWFWVRQVSGGGSGGGSGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2155	1-6	1-2	0.2	0.004

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved (+) with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs). Conc. = concentration; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

E. BIACore Analysis

For BIACore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 µg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

- 5 Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT
 10 completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides, and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block
 15 the insulin-binding site. The results are shown in Tables 12 and 13. The amino acid sequences referred to in the tables are identified in Figures 8 and 9A-9B, except the 447 and 2A9 sequences, which are shown below.

TABLE 12

BIACore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 µM)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 µM) + IR	43	Formula 6 Motif
	D8-MBP (1.6 µM) + IR	56	Formula 6 Motif
	D10-MBP (3.4 µM) + IR	81	Formula 11 Motif
	447-MBP (11.5 µM) + IR	195	hGH Pept. Fus.
	MBP (13 µM) + IR	209	Negative Control

20

The A7 (20A4), D8, and D10 peptide sequence are shown in Figures 8 and 9A-9B. The 447 peptide sequence is: LCQRLGVGVWPGWLSGWCA (SEQ ID NO:2156).

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TABLE 13

BIAcore Results – Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR + D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

5 The concentration of each peptide was about 40 µM and the concentration of IR was 450 nM. The 20D1, 20A4, and D8 peptide sequences are shown in Figures 8 and 9A-9B. The remaining peptide sequences are as follows: 447 = LCQRLGVGVPGWLSGWCA (SEQ ID NO:2156); 2A9 = LCQSWGVRIGWLTLCP (SEQ ID NO:2157); 20C11 = DRAFYNGLRLDLVGAVYGAWD (SEQ ID NO:1659); H2 = VTFTSAVFHENFYDWFWVRQVS (SEQ ID NO:1784).

10

Regarding preparation of a Site 1 agonist comprising two H2C peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region (Figure 29).

15

F. Stimulation of Autophosphorylation of IR by MBP-Fusion Peptides

MBP fusion peptides were prepared as described above, and then assayed for autophosphorylation of a insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993; clone 969) (see Example, above). The results of these experiments shown in Figure 30 indicate that the H2C monomer and H2C-H2C homodimer peptides stimulate autophosphorylation of IR *in vivo*. H2C dimer peptides (Site 1-Site 1) with a 6 amino acid linker (H2C-6aa-H2C) were most active in the autophosphorylation assay. Other active dimer peptides are also shown in Figure 30, particularly H2C-9aa-H2C, H2C, H2C-12aa-H2C, H2C-3aa-H2C, and F8.

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**G. Insulin Receptor Binding Affinity and Fat Cell Potency of
MBP-Fusion Peptides**

Results of assays to determine binding affinity for insulin receptor and fat cell potency of the MBP-fusion peptides are shown in Table 14, below.

TABLE 14

Pepptide	SEQ ID NO:	Formua	Site	Sequence	HIR Kd (moff)	FFC
RB426	2158	F6	2	MBP...NNNLGIEGRISEFIEGR AQPAMA WLQEWAIVQCEVYGRGCP S AAA(ETAG)AA	1.4*10 ⁻⁶	
RB429	2159	F6-F6	2-2	MBP...NNNLGIEGRISEFIEGR AQPAMA WLQEWAWVQCEVYGRGCP SGGSKWLDQEWAIVQCEVYGRGCP SAA(ETAG)AA	1.3*10 ⁻⁶	
RB505M	2160	F4	2	MBP...NNNLGIEGRISEFIEGR DYYKDDDK HCVLEELFWGASLFGYCSCAAA(ETAG)AA		
RB517M	2161	F4-F4	2-2	MBP...NNNLGIEGRISEFIEGR DYYKDDDK HCVLEELFWGASLFGYCSCGGGGS HCVLEELFWGASLFGYCSCGAAA(ETAG)AA		
RB515	2162	F4-F4	2-2	MBP...NNNLGIEGRISEFIEGR DYYKDDDK HCVLEELFWGASLFGYCSCGGGGS HCVLEELFWGASLFGYCSCGGGGS		
RB510	2163	F4-F1-F4	2-2-2	MBP...NNNLGIEGRISEFIEGR DYYKDDDK HCVLEELFWGASLFGYCSCGGGGS HCVLEELFWGASLFGYCSCGGGGS HCVLEELFWGASLFGYCSCGGGGS		
RB437	2164	F1	1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSAAA(ETAG)AA	8.3*10 ⁻⁶	
RB463	2165	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSQSGSFHENFYDWFVRCVSAAA(ETAG)AA	9.6*10 ⁻⁷	
RB439	2166	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSQSGSGSFHENFYDWFVRCVS-ETAG	5.5*10 ⁻⁷	
RB436	2167	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSQSGSGSFHENFYDWFVRCVSAAA(ETAG)AA	4.1*10 ⁻⁷	
RB449	2168	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSQSGSGSGGS AQPAMAFHENFYDWFVRCVSAAA(ETAG)AA	6.2*10 ⁻⁷	
RB435	2169	F1-F1-F1	1-1-1	MBP...NNNLGIEGRISEFIEGR AQPAMA FHENFYDWFVRCVSQSGSFHENFYDWFVRCVSQSGSFHENFYDWFVRCVSAAA(ETAG)AA	2.0*10 ⁻⁶	
RB502	2170	F1	1	MBP...NNNLGIEGRISEFIEGR DYYKDDDK VRVDWLQRNANFYDWFVVAELVAAA(ETAG)AA		
RB508M	2171	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR DYYKDDDK VRVDWLQRNANFYDWFVVAELGGGGSGRVDWLQRNANFYDWFVVAELGAAA(ETAG)A		
RB509M	2172	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR DYYKDDDK VRVDWLQRNANFYAWFVAELGGGGSGRVDWLQRNANFYDWFVVAELGAA A(ETAG)AA		
RB452	2173	F1	1	MBP...NNNLGIEGRISEFIEGR AQPAMARGGGTYEMFESALRKHGAGAAA(ETAG)AA	7.8*10 ⁻⁷	
RB427	2174	F1-F1	1-1	MBP...NNNLGIEGRISEFIEGR AQPAMARGGGTYEMFESTLRKHGAGGGSGSGGTTYEWFESALRKHGAGAAA(ETAG)AA	3.3*10 ⁻⁶	
RB434	2175	F1-F1	1-1	MBP...NNNLGIEGRISEFIEVR AQPAMA RGGGTYEWFESALRKHGAGAAA(ETAG)AA	3.2*10 ⁻⁶	

RB513	2176	F1	1	MBP .NNNNLIGEGRISEFIEGRDYKDDDKGSIDESFYDWFERQLGKAAA(ETAG)AA
RB516	2177	F1-F1	1-1	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKGSIDESFYDWFERQLGKGGSGS3IDESFYDWFERQLGKAAA(ETAG)AA
RB512	2178	F1-F1	1-1	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSAAA(ETAG)AA
RB464	2179	F1	1	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSAAA(ETAG)AA
RB446	2180	F4	2	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSAAA(ETAG)AA
RB459	2181	F3	1	MBP ...NNNNLIGEGRISEFISADYKDDDLRMRYFEERPSLAAA(ETAG)AA
RB430	2182	F1-F3	1-1	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSGGSGS LDALDRMLRYFEERPSLETAG
RB430	2183	F1-F3	1-1	cleaved DYKDDDKFHENFYDWVRQVSGGSGSLDALDRMLRYFEERPSLAAA(ETAG)AA
RB431	2184	F1-F4	1-2	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSGGSGS HLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB431	2185	F1-F4	1-2	cleaved DYKDDDKFHENFYDWVRQVSGGSGSHLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB432	2186	F1-F4	1-2	MBP -NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSGGSGS HLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB432	2187	F1-F4	1-2	cleaved DYKDDDKFHENFYDWVRQVSGGSGGGHLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB433	2188	F1-F4	1-2	MBP ...NNNNLIGEGRISEFIEGRDYKDDDKFHENFYDWVRQVSGGSGGSS HLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB508	2189	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYDWVVAELGGSGGSGRVDWLQRNANFYDWVVAELGAAGAPVPDPLEPRSA
RB509	2190	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYAWFVVAELGGSGGSGGGSGRVDWLQRNANFYDWVVAELGAAGAPVPDPLEPRSA
RB505	2191	F4	2	DYKDDDDKHLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB517	2192	F4-F4	2-2	DYKDDDDKHLCVLELFWGASLFGYCSCGAAA(ETAG)AA
RB521	2193	F1-F1	1-1	MADYKDDDDKGSIDESFYDWFERQLGKGGSGS3IDESFYDWFERQLGKAAA(ETAG)PG
RB535	2194	F1-F1	1-1	MADYKDDDDKGSIDESFYDWFERQLGKGGSGS3IDESFYDWFERQLGKAAA(ETAG)PG
RB540	2195	F6	2	MADYKDDDKWLDQEWAQVCEVYGRGCPAAA(ETAG)PG
RB539	2196	F6-F1	2-1	MADYKDDDKWLDQEWAQVCEVYGRGCPAAA(ETAG)PG
RB537	2197	F1-F6	1-2	MADYKDDDKGSIDESFYDWFERQLGKGGSGS3WLDQEWAQVCEVYGRGCPAAA(ETAG)PG
RB538	2198	F1-F6	1-2	MADYKDDDKGSIDESFYDWFERQLGKGGSGS3WLDQEWAQVCEVYGRGCPAAA(ETAG)PG
RB626	2199	F6-F1	2-1	MADYKDEIAEWGGRVCLYGRCVGGGSGSGSGSGSGS3IDESFYDWFERQLGKAAA(ETAG)PG
				3.0*10^-10 +++

RB625	2200	F6-F1	2-1	MADYKDDDKWLDQEWAQCEVYGRGCPSQPPPDTTHRADPQGSLSDESFYDWFERQLGKAAA(ETAG)PG	3.8*10^-10	+++++
RB622	2201	F6-F1	2-1	MADYKDDDKWLDQEWAQCEVYGRGCPSTPKPPTFPPLSADGSLDESFYDWFERQLGKAAA(ETAG)PG	1.0*10^-9	+++
RB596	2202	F1	1	MQNDGSLDESFYDWFERQLGHHHHHHPG	9.4*10^-8	
RB569	2203	F1	1	MGSLDESFYDWFERQLGEEEGGDHHHHHHPG	2.1*10^-7	
RB570	2204	F1	1	MQNDGSLDESFYDWFERQLGEEEQGDHHHHHHPG	2.5*10^-8	

ETAG = GAPVVPYPDPLEPR(SEQ ID NO: 2205); MBP...NNNNL = fusion junction to MBP at c-terminus of MBP; All dimers are linked C-N.

Example 9: *In Vivo Assays for Insulin Agonists*

To test the *in vivo* activity of dimer peptide S519, an intravenous blood glucose test was carried out on Wistar rats. Male Mol:Wistar rats, weighing about 300 g, were divided into two groups. A 10 µl sample of 5 blood was taken from the tail vein for determination of blood glucose concentration. The rats were anaesthetized with Hypnorm/Dormicum at t = -30 min and blood glucose was measured again at t = -20 min and at t = 0 min. After the t = 0 sample was taken, the rats were injected into the tail vein with vehicle or test substance in an isotonic aqueous buffer at a 10 concentration corresponding to a 1 ml/kg volume of injection. Blood glucose was measured at times 10, 20, 30, 40, 60, 80, 120, and 180 min. The Hypnorm/Dormicum administration was repeated at 20 minute intervals. 15 Results shown in Figure 33 demonstrate that the S519 (at 20 nmol/kg) peptide lowered blood glucose levels similar to levels observed for human insulin (at 2.5 nmol/kg) (n=8). The S519 peptide and human insulin showed comparable *in vivo* effects, both in magnitude and onset of response (Figure 33).

Example 10: IGF-1 Surrogates

Three major groups of peptide IGF-1 surrogates were obtained from 20 IGF-1R panning experiments: Site 1 A6 (FyxWF) (SEQ ID NO: 1596); Site 1 B6 (FyxxLxxL) (SEQ ID NO: 1732), and Site 2 (C-C looped). See Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000. Active surrogates included 20E2 and RP6 (B6-like; Formula 2), S175 (A6-like; Formula 1), G33 (A6-like; Formula 1), RP9 (A6-like; Formula 1), D815 25 (Site 2), and D8B12 (Site 2) peptides. The IGF-1 surrogates were analyzed by various assays, described as follows.

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A. Phage Competition

Phage competition studies were performed with Site 1 (RP9) and Site 2 (D815) monomer peptides. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixtures were incubated for 2 hr at room temperature. Plates were washed three times with PBS and 100 µl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated at room temperature for 60 min. After washing, 100 µl of ABTS was added per well and the plates read in a microtiter reader at 450 nM.

Phage included RP9 (A6-like; Formula 1); RP6 (B6-like; Formula 2); D8B12 (Site 2); and D815 (Site 2). Peptides included RP9 and D815.

15

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D8B12	6	2	WLEQERAWIWCEIQGSGCRA	1884
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
RP6	2	1	TFYSCLASLLTGTPQPQRGPWERCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559

Results shown in Figures 34A-34E demonstrate that that RP9 and D815 peptides competed both Site 1 and Site 2 phage. These results illustrate the allosteric nature of the interaction with IGF-1R.

20 Phage competition studies were also performed with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixture

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incubated for 2 hr at room temperature. Plates were washed three times with PBS and 100 μ l of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated for 60 min at room temperature. After washing, 100 μ l of ABTS was added per 5 well and the plates read in a microtiter reader at 450 nM. Phage included RP9, RP6, D8B12, and D815. Peptides included D815-6L-RP9 and D815-12L-RP9. Linker sequences are underlined and shown below.

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D815-6L-RP9	6-1	2-1	LDQERAWLWCEISGRGCLSGGS GGSGS GLDESFYDWFERQLGK K	2207
D815-12L-RP9	6-1	2-1	WLDQERAWLWCEISGRGCLSGGS GGSGS GGSGS GG SLDESFYD WFERQLGKK	2208

10 D8B12, D815, RP6, and RP9 amino acid sequences are shown in the previous section. Results shown in Figures 35A-35E demonstrate that dimers competed both Site 1 and Site 2 phage. This indicates that both dimer units were active at IGF-1R.

B. IGF-1 Proliferation Assays

15 FDCP-2 cells expressing the IL-3 and human IGF-1R receptors were grown in RPMIk-1640 medium supplemented with 15% fetal bovine serum (FBS) and 5% WEHI conditioned medium (containing IL-3) in accordance with routine methods. Prior to an experiment, the cells were pelleted and washed two times in PBS. Following this, cells were resuspended in RPMI-1640 medium with 2% FBS and added to a 96-well plate at a concentration 20 of 2×10^4 cells/well in 75 μ l. This was designated as the cell plate.

Peptides were suspended in PPMI-15% FBS (test medium). For the agonist assay, medium was added to rows 2-12 of a 96 well plate. The peptide was added to row 1 in 200 μ l of test medium at a final concentration of 60 μ M. The peptide was serially diluted (1:1) across rows 2-11. No 25 peptide was added to row 12 (control; cells without IGF-1). For the

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antagonist assay, test medium containing 10 ng/ml IGF-1 (ED₅₀ test medium) was added to all wells of a 96 well plate. To row 1 was added 100 µl of peptide in ED₅₀ test medium at a concentration of 120 µM. The peptide was serially diluted (1:1) across rows 2-11. No peptide was added to row 12
5 (control; cells with IGF-1).

For both agonist and antagonist assays, 75 µl from the working plates was transferred to the appropriate rows in comparable cell plates. The starting peptide concentration for both agonist and antagonist assays was 30 µM. Each peptide was done in duplicate. Plates were incubated at 37°C
10 for 45-48 hr. Ten microliters of WST-1 (Cell Proliferation Reagent, Roche cat # 1 644 807) were added to each well and the plates were read in an ELISA reader (440/700 dual wavelength) each hour for 4 hr. Graphs were prepared from the raw data using Sigma Plot. Peptides included:

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
20E2	2	1	DYKDFYDAIDQLVRGSARAGGTRD	2209
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
G33	1	1	GIISQSCPESFYDWFGQVSDPWWCW	1600
RP6	2	1	TFYSCLASLLTGTPQPNRGPWERCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559
S175	1	1	GRVDWLQRNANFYDWFVAELG	1560

15

Results of the IGF-1 proliferation assays are shown in Figures 36-42. Figure 36 demonstrates that peptides G33 (Site 1; ED₅₀ ~ 10 µM) and D815 (Site 2; ED₅₀ ~ 2 µM) showed agonist activity at IGF-1R, whereas peptides RP9 and RP6 showed no agonist activity. Figure 37 demonstrates
20 that peptides RP6 (Site 1; ED₅₀ ~ 1 µM) and RP9 (Site 1; ED₅₀ ~ 7 µM) showed antagonist activity at IGF-1R, whereas peptides G33 and D815 showed no antagonist activity. Figure 38 demonstrates that peptides S175 and 20E2 exhibited weak agonist activity at IGF-1R (ED₅₀ > 10 µM). Figure

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39 shows that D815-RP9 dimers with 6- or 12-amino acid linkers acted as agonists at IGF-1R. Figure 40 shows that dimer peptide D815-6-G33 was inactive as an agonist at IGF-1R. Figure 41 shows that monomer peptide RP6 acted as an antagonist at IGF-1R. The IGF-1 standard curve
5 determined for FDCP-2 cells is shown in Figure 42.

The IGF-1R data for the Site 1 and Site 2 peptides is summarized in Table 15, below.

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TABLE 15

Mon./Dimer	Form.	Site IGF-1R	Link.	Sequence	SEQ ID NO:	nM Ki app Kd	nM ED ₅₀ Growth	Max Action	nM IC ₅₀ Antag.	Ki/ ED50	Class
IGF-1			NA			0.69	0.30	100	2	2.3	A
rG33	1	1	NA	<u>GIISSQSCPESFYDWFAAGQVSDPWWCW</u>	1600	1450	500	>50	—	2.9	A
rD815	6	2	NA	<u>WLDQERAWLWCEISGRGCLS</u>	2206	4080	500	>50%	—	8.2	A
RP9	1	1	NA	<u>GSLIDESFYDWFERQLG</u>	1559	417	—	<10%	900	0.5	N
D815-G33	6-1	2-1	6 aa	<u>WLDQERAWLWCEISGRGCLSGGGSGIIISQSCPESF</u> <u>YDWFAGQVSDPWWCW</u>	2210	624	—	<10%	nd	nd	
D815-RP9	6-1	2-1	6 aa	<u>WLDQERAWLWCEISGRGCLSGGGSGSGLDESFYDW</u> <u>FERQLGKK</u>	2211	36	50	>50%	>500	0.8	A
D815-RP9	6-1	2-1	12 aa	<u>WLDQERAWLWCEISGRGCLSGGGSGGGSGSGL</u> <u>DESFYDWFERQLGKK</u>	2212	3	10,000	100	—	0.0003	A

A = agonists; N = antagonist; nd = not determined; NA = not applicable; Form. = formula; Mon. = monomer; Antag. = antagonist; Link. = linker; Linker sequences are underlined.

Example 11: Panning Peptide Libraries**A. Panning IGF-1 Surrogate Secondary Libraries**

Soluble IGF-1R ("sIGF-1R") was obtained from R&D Systems. The soluble protein (> 95% pure) included the heterotetrameric (alpha 2-beta 2) extracellular domain of IGF-1R isolated from a mouse myeloma cell line. sIGF-1R (500 ng/well) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, NUNC) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h. Eight wells were used for each round of panning for the G33 and RP6 secondary libraries. The phage were incubated with MPBS for 30 min at RT, then 100 µl was added to each well.

For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 µl/well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30° C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80° C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones was picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones determined the amino acid sequences summarized in Figure 43A-43B.

B. Panning Peptide Dimer Libraries

Microtiter plates were coated and blocked by standard methods, as follows. Plates were coated with sIGF-1R (see Example, above) or soluble IR (Bass construct; Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375) in

5 0.2 M NaHCO₃, pH 9.4. One hundred microliters of solution containing either 50 ng IR or IGF-1R (rounds 1 and 2), 25 ng IR or IGF-1R (round 3), or 12.5 ng IR or IGF-1R (round 4) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nalge NUNC) and incubated overnight at 4°C. Wells were then blocked with a solution of 2%

10 non-fat milk in PBS (MPBS) at RT for at least 1 h.

Eight wells coated with IR or IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was 3×10^{13} cfu/ml. For subsequent rounds, the input phage titer was approximately 10^{12} cfu/ml. Phage were

15 incubated for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS. Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 hr at 37°C prior

20 to the addition of helper phage, ampicillin, and glucose (2% final concentration).

After incubation for 1 hr at 37°C, the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. Phage amplified overnight were

25 then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity. Several clones from each pan were further tested for binding to IR or IGF-1R in phage ELISA by competition with soluble peptides as described in Beasley *et al.* International Application PCT/US00/08528, filed

30 March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000. Competition was performed by addition of 5 µl of RP9

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peptide, recombinant D8 peptide, or both per well, followed by addition of 100 µl of phage per well. Representative peptides are shown in Figures 44A-44B and in Table 16, below.

TABLE 16

Pep.	SEQ ID NO:	Form.	Site IR	Sequence	Description
RP27	2213	6-1	2:1	GLDQEQQAWVECEVYGRGCPYGSLSDESFYDWFERQLG	No linker
RP28	2214	6-1	2:1	RLEEEWAQWVQCEVYGRGCPSSGGSGSLSDESFYDWFERQLG	EEE Stretch in D8
RP29	2215	6-1	2:1	SLDREWAVKCEVYGRGCPGCCGGSGSLSDESFYDWFERQLG	Repeat isolate
RP30	2216	6-1	2:1	SLEEEWAQVECEVYGRGCPSSGGSGSLSDESFYDWFERQLG	D8 by Design
RP31	2217	6-1	2:1	SLEEEWAQVECEVYGRGCPSSGGSGSLLDESFYHWFDRQLR	D8 & RP9 by design
RP32	2218	6-1	2:1	SIEEEWAQIKCDWIGRCPPQQGSGSGLLDESFYHWFDRQLR	D8 & RP9 by design
RP33	2219	6-1	2:1	QLDLEWAQWVQCEVYGRGCGGSGSLSDESFYDWFERQLG	3 amino acid linker
RP34	2220	6-1	2:1	QLDEEWAGVQCEVYGRGCSLDESFYDWFERQLG	No linker
RP35	2221	6-1	2:1	RLEEEWRWVQCEVYGRGCAAGGSGSGGSGSLSDESFYDWFERQLG	EEE Stretch in D8
RP36	2222	6-10	2:1	SLDQEWAQWVQCEVYGRGCPSSGGSGSLSDWAGYEWFEQLD	D8 (W1->S)-Group 6 by design

Pep. = peptide; Form. = formula; Linker sequences are shown in bold and underlined; All dimers are linked C-N

C. Determination of Amino Acid Preferences

For both monomer and dimer peptides, amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency >2-fold was considered preferred. Most preferred amino acid(s) were those that have the greatest fold enrichment after panning. Preferred amino acid sequences for RP9, D8, and Formula 10 (Group 6) peptides are shown below.

TABLE 17

Peptide	Sequence	SEQ ID NO:
RP9	GSLDESFYDWFERQLG	1559
Regular	G L A D E D F Y E W F ER Q LR L	2223
w/ Peptide	G OL D ED F Y E W F DRQLS A	2224
w/ Insulin	G FM D ES F Y E W F ER Q LR W A	2225

Table 17 shows preferred amino acid sequences for RP9 peptides. Residues in bold indicate strong preference; underlined residues indicate positions where more than one amino acid preference is seen. The first column indicates the conditions used for the panning procedure. "RP9" indicates sequence of the parent RP9; "Regular" indicates regular pan as described in methods for panning of random libraries; "w/ peptide" indicates panning in the presence of 2 nM RP9 peptide; "w/ insulin" indicates panning in the presence of 2 nM insulin.

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TABLE 18

Peptide	Sequence	SEQ ID NO:
D8 Parent:	WLDQEWA <u>WVQCEVYGRGCPs</u>	2129
Dimer Consensus	sLEEEWaQIECEVY/ <u>WGRGCps</u>	2226
Monomer Consensus	sLEEEWaQIqCEIY/ <u>WGRGCry</u> W	1548

Table 18 shows preferred amino acid sequences for D8 peptides. Upper case residues in bold indicate strong preference (>90% frequency); 5 upper case letters, non-bold, indicate some preference (5-15% higher frequency than expected); lower case letters indicate less preference (2-5% higher frequency than expected); similar preferences seen in D8 in both monomer and dimer libraries. The underlined Y/W indicates that both residues are equally preferred at that position. In the original D8 sequence 10 that position is occupied by Y.

TABLE 19

Peptide	Sequence	Type	SEQ ID NO:
Group 6	W (A/E) GYEW (F/L)	preferred core	1549
Group 6	DSDWAGYEW <u>FEEQLD</u>	preferred sequence	1595

Table 19 shows preferred amino acid sequences for Group 6 peptides. Underlined residues indicate preferred N-terminal and C-terminal 15 extensions.

Example 12: Fluorescence-Based IGF-1R Binding Assays

A. Heterogeneous Time-Resolved Fluorometric Assays

The effect of recombinant peptide surrogate G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant

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human IGF-1R (rhIGF-1R) was determined using heterogeneous time-resolved fluorometric assays (TRF; DELFIA[®], PE Wallac, Inc.). The rhIGF-1R protein included the extracellular domain of the receptor pre-propeptide, up to amino acid residue 932 (A. Ullrich et al., 1986, *EMBO J.* 5:2503-2512).

- 5 Duplicate data points were collected at each concentration of competitor and the lines were designed to represent the best fit to a four-parameter non-linear regression analysis ($y = \min + (\max-\min)/(1+10^{((\log IC_{50}-x)*Hillslope)})$) of the data, which was used to determine IC₅₀ values.

The assay was performed using a 96-well clear microplate (NUNC
10 MaxiSorp) with a final volume of 100 µl. Microtiter plates were coated with 0.1 µg rhIGF-1R in 100 µl of NaHCO₃, pH 8.5 buffer, and incubated overnight at room temperature (RT). The plates were washed 3-times with 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl (TBS). This was followed by addition of 200 µl blocking buffer (TBS containing 0.05%
15 Bovine Serum Albumin (BSA, Cohn Fraction V)), and incubated for 1 hr at RT. The plates were washed 6-times with a 1 X solution of Wallac's DELFIA[®] wash concentrate. Competitor was added in a volume of 50 µl and serially diluted across the microtiter plate in TBS containing 0.05% BSA.
Non-specific binding (background) was determined in the presence of 60 µM
20 hIGF-1.

Fifty microliters of b-rhIGF-1, 10 nM, diluted in TBS containing 0.05% BSA was added. The plates were incubated for 2 hr at RT. After incubation, plates were washed 6-times with a 1X solution of Wallac's DELFIA[®] wash concentrate. Then the plates were treated with 100 µL of
25 Wallac's DELFIA[®] Assay Buffer containing a 1:1000 dilution of europium-labeled streptavidin and incubated for 2 hours at RT. This was followed by washing 6-times with a 1 X solution of Wallac's DELFIA[®] wash concentrate. One hundred microliters of Wallac's DELFIA[®] enhancer was added, and the plates were shaken for 30 min at RT. After shaking, the fluorescence signal
30 at 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.).

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Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al., 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~20. The results of these 5 experiments are shown in Figure 45. The IC_{50} value calculated for rG33 is shown in Table 20, below.

The effect of recombinant peptide surrogates D815 (rD815), RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 on the binding of b-rhIGF-1 to rhIGF-1R was determined using the fluorometric assay described 10 above. IGF-1 was used as a control. Duplicate data points were collected at each concentration of competitor and the lines represent the best fit to a four-parameter non-linear regression analysis, which was used to determine IC_{50} values. Results for rD815 are show in Figure 46; results for RP9 are shown in Figure 47; results for D815-6-G33 are shown in Figure 48; results 15 for D815-6-RP9 are shown in Figure 49; and results for D815-12-RP9 are shown in Figure 50; the results for IGF-1 are shown in Figure 51. The IC_{50} values for the rD815, RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 peptides, and IGF-1 are shown in Table 20, below. Linker sequences are underlined.

20

TABLE 20

Competitor	Sequence	SEQ NO:	IC_{50} (M)
rG33	GIISQSCPESFYDWFA <u>GQVSDPWW</u> CW	1600	1.45×10^{-6} M
rD815	WLDQERAWLWCEISGRGCLS <u>GGSGGSG</u> GIIS	2206	4.08×10^{-8} M
RP9	GSLDESFYDWFERQLG	1559	4.17×10^{-7} M
D815-6aa-G33	WLDQERAWLWCEISGRGCLSG <u>GGSGGSG</u> GIIS	2210	6.24×10^{-7} M
D815-6aa-RP9	WLDQERAWLWCEISGRGCLSG <u>GGSGGSG</u> SGL DESFYDWFERQLGKK	2211	3.57×10^{-8} M
D815-12aa-RP9	WLDQERAWLWCEISGRGCLSG <u>GGSGGSG</u> GG SGGSGS <u>LDESFYDWFERQLGKK</u>	2212	3.22×10^{-9} M
IGF-1			6.85×10^{-10} M

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The order of potency of all peptides or dimers compared to IGF-1 was determined as: IGF-1 > D815-12aa-RP9 >> D815-6aa-RP9 > RP9 \cong D815-6aa-G33 > rG33 > rD815. These results suggest that the coupling of D815 with RP9 using an extended linker (12 versus 6 amino acids) produced a potent competitor that approximates the affinity of IGF-1 for its own receptor.

B. Time-Resolved Fluorescence Resonance Energy Transfer Assays

The effect of Site 1 peptide surrogates, Site 2 peptide surrogates, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R was determined using time-resolved fluorescence resonance energy transfer assays (TR-FRET). Best fit non-linear regression analysis of the data, was used to determine dissociation rate constants. Each data point represents a single observation.

The assay was performed using a 96-well white microplate (NUNC) with a final volume of 100 μ l. Final incubation conditions were 16.5 nM b-20E2, 2.2 nM SA-APC (streptavidin-allophycocyanin), 2.2 nM Eu³⁺-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1 % BSA (Cohn Fraction V). Reactions were allowed to reach equilibrium for 6 hr at RT. Following this, various peptide surrogates or IGF-1 were added at a final concentration of 100 μ M or 30 μ M, respectively. The addition of peptides or IGF-1 initiated the measurement of dissociation (Time Zero, sec). The fluorescence signal at 665 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.) at 30 sec intervals.

Results of these experiments are shown in Figure 52. The buffer controls did not vary over the time interval of study, which demonstrated that the equilibrium was not disturbed by the addition of diluent at Time zero. The addition of excess (> 1000-fold 20E2 K_d for IGF-1R) Site 1 peptides such as H2C, 20E2, or RP6 did not differ depending on specific the peptide used, and the dissociation rates of b-20E2 were similar for these peptides. D8B12 (Site 2 peptide) and IGF-1 (binds both Site 1 and Site 2) did

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demonstrate significant differences in the rate of dissociation of b-20E2. This would suggest that these agents act as non-competitive or allosteric regulators of Site 1 binding.

The effect of various peptide surrogates or peptide dimers on the
 5 binding of biotinylated-20E2 (B-20E2) to recombinant human IGF-1R was determined using TR-FRET assays, described above. For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis ($y = \text{min} + (\text{max}-\text{min})/(1+10^{(\log IC_{50}-x)*\text{Hillslope}})$) of the data,
 10 which was used to determine IC_{50} values.

The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μl . Final incubation conditions were 15 nM b-20E2, 2 nM SA-APC, 2 nM Eu³⁺-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1
 15 % BSA (Cohn Fraction V). After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al,
 20 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~ 4. Results of these experiments are shown in Figure 53. Table 21, below, shows the IC_{50} values calculated for these experiments. Notably, the C1 peptide showed IGF-1R affinities of ~1 nM (Figure 53) and ~10 nM (Table 21) in these assays.

25

TABLE 21

Competitor	Sequence	SEQ ID NO:	Formula	Site IGF-1R	IC_{50} (M)
C1	CWARPCGDAANFYDWFWQQAS	1550	1	1	8.80E-10
IGF-1					2.93E-09
RP9	GSLDESFYDWFERQLG	1559	1	1	3.93E-08

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20E2	DYKDFYDAIDQLVRG SARAGGTRD	2209	2	1	1.04E-07
E8	GGTVWPGYEWL RNA	2118	10	2	2.53E-07
H2C	FHENFYDW FVQR VSKK	2117	1	1	4.60E-07
S173	LDAL DRLM RYFE ERPSL	1830	3	1	6.29E-06
D8B12	WLEQERAWI WCEI QGS GCRA	1884	6	2	1.13E-05
A6	SAKNFYDW FVKK	1551	1	1	3.10E-05

C. Fluorescence Polarization Assays

- The effect of various peptide monomers and dimers on the binding of fluorescein-RP-9 (FITC-RP9) to soluble human insulin receptor-immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375) was determined using fluorescence polarization assays (FP). For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC₅₀ values.
- The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 µl. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05 % BGG (bovine gamma globulin), 0.005 % Tween-20®. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 (Z' = 1-(3σ₊+3σ₋)/|μ₊-μ₋|; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73) and the assay dynamic range was ~125 mP. In parallel with these experiments, TR-FRET assays were performed using rhIGF-1R and b-20E2, as described above. Results of the FP and TR-FRET experiments are shown in Table 22, below.

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TABLE 22

Peptide	FP sIR-Fc	TR-FRET rhIGF-1R	Binding Ratio IGF-1R / IR	Formula	Site IGF-1R	SEQ ID NO:	Sequence
RP4	17	8100	476	2	1	1552	PPWGARFYDAIEQLVFDNL
S175	10	1650	165	1	1	1560	GRVDWLQRNANFYDWFVA ELG
RP15	28	706	25	1	1	2130	SQAGSAFYAWFDQVLRTV
H2C (D117)	66	600	9	1	1	2117	FHENFYDWFVQRVSKK
20E2 (D118)	51	100	1.9	2	1	2209	DYKDFYDAIDQLVRGSARA GGTRD
RP9	24	33	1.4	1	1	1559	GSLDESFYDWFERQLG
G33	139	178	1.3	1	1	1600	GIISQSCPESFYDWFAGQV SDPWWCW
E8 (D120)	206	175	0.85	10	2	2118	GGTVWPGYEWLRNA
C1 (D112)	52	10	0.19	1	1	1550	CWARPCGDAANFYDWFV QQAS
RP16	6400	961	0.15			1553	VMDARDDPFYHKLSELVT

FP sIR-Fc column shows IC₅₀ (nM) values obtained (vs. FITC-RP9); TR-FRET rhIGF-1R column shows IC₅₀ (nM) values obtained (vs. b-20E2); for Binding Ratio: higher values indicated higher affinity for IR than IGF-1R.

These results demonstrated that S175, RP4, and RP15 showed high affinities for IR and showed high binding ratios for IGF-1R over IR. H2C, 20E2, RP9, and C1 were slightly less potent than S175, RP4, and RP15 at IR, and these peptides had lower binding ratios for IGF-1R over IR. G33 and E8 were less potent than S175, RP4, and RP15 at IR, and showed comparable binding to IGF-1R and IR. RP16 had poor potency at IR and IGF-1R, but had higher affinity for IGF-1R than IR.

Example 13: Insulin Receptor Surrogates with Enhanced Specificity

Peptide S597 was tested for its bioactivity relative to insulin. SGBS cells (a human adipocyte cell line) were incubated with various concentrations of human insulin or peptide S597 and cellular uptake of ¹⁴C-glucose was measured essentially as described in Example 4. The results

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(as illustrated in Figure 54) indicate that the potency of S597 in stimulating glucose uptake is at least as good as that of human insulin.

The glucose-lowering effect of peptide S597 and peptide S557 in rats was compared with that of insulin as follows: Eighteen male Wistar rats,

5 200-225 g, fasted for 18 h, were anesthetized using Hypnorm-Dormicum (1.25 mg/ml Dormicum, 2.5 mg/ml fluanisone, 0.079 mg/ml fentanyl citrate) 2 ml/kg as a priming dose 30 min prior to test substance dosing and additional 1 ml/kg every 20 minutes (at time points -10 min, 10 min and 30 min relative to test substance dosing).

10 The rats were allocated into three groups. The animals were dosed with an intravenous injection (tail vein), 2 ml/kg, of either human insulin 1.25 nmol/kg (n=6) or S557 peptide 5 nmol/kg (n=6) or S597 peptide 5 nmol/kg (n=6). Blood samples for the determination of whole blood glucose concentration were collected in heparinized 10 µl glass tubes by puncture of 15 the capillary vessels in the tail tip at times -20 min and 0 min (before dosing), and at times 10, 20, 30, 40, 60, 80, 120, and 180 min after dosing. Blood glucose concentrations were measured after dilution in analysis buffer by the immobilized glucose oxidase method using an EBIO Plus autoanalyzer (Eppendorf, Germany).

20 The results (as illustrated in Figure 55) indicate that the blood glucose lowering effect of S597 in rats is about 4 times lower than that of human insulin. The improved effect of S597 relative to S557 shows the effect of N-terminal acetylation.

25 The glucose-lowering effect of different concentrations of peptide S597 was also tested by intravenous administration to fasted Goettingen minipigs weighing about 15 kg. The results (as illustrated in Figure 56) indicate that the glucose-lowering effect at 3 nmol/kg S597 is comparable to that of 0.3 nmol/kg human insulin.

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Example 14: Co-administration of Therapeutic Peptides

The rate of disappearance of two co-administered peptides was tested as follows:

Mixtures containing 600 nmol/ml peptide S557 and 1800 nmol/ml B²⁹-

- 5 N -(N-lithocetyl- -glutamyl)-des(B30) human insulin included ¹²⁵I-labeled peptides were injected into the neck of a pig. Radioactivity at the injection site was monitored over time using an external gamma counter.

The results (as illustrated in Figure 57) indicate that the disappearance of either peptide was not influenced by the presence of the
10 second peptide.

Incorporated herein by reference in its entirety is the Sequence Listing for the application, comprising SEQ ID NO:1 to SEQ ID NO:2227. The Sequence Listing is disclosed on three CD-ROMs, designated "CRF",

- 15 "Copy 1", and "Copy 2". The Sequence Listing is a computer-readable ASCII file named "18784057PC.app.txt", created on September 23, 2002, in IBM-PC machine format, on a MS-Windows®98 operating system. The 18784057PC.app.txt file is 927,476 bytes in size.

20 As various changes can be made in the above compositions and methods without departing from the scope and spirit of the invention, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

25

The contents of all patents, patent applications, published articles, books, reference manuals, texts and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

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WHAT IS CLAIMED IS:

1. A method of decreasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence in an amount sufficient to decrease insulin activity, wherein the amino acid sequence comprises a subsequence that comprises a sequence that binds to Site 1 of insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin receptor, and wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 1 to Site 2, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.
2. The method according to claim 1, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
3. The method according to claim 2, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.

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4. The method according to claim 2, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
5. The method according to claim 2, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
6. The method according to claim 2, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCPS (SEQ ID NO:2129).
7. The method according to claim 2, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
8. The method according to claim 2, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NOS:926-1061 (Figures 3A-3E); SEQ ID NOS:1244-1253 (Figure 9A); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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9. The method according to claim 2, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).
10. The method according to claim 2, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).

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11. The method according to claim 2, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117:

FHENFYDWFVRQVSKK (SEQ ID NO:1556);

FHENFYDWFVRQVS (SEQ ID NO:1557);

RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);

GSLDESFYDWFERQLG (SEQ ID NO:1559);

GLADEDFYEWFERQLR (SEQ ID NO:1561);

GLADEFYEWFDRQLS (SEQ ID NO:1562);

GQLDEDFYAWFDRQLS (SEQ ID NO:1563);

GQLDEDFYAWFDRQLS (SEQ ID NO:1564);

GFMDESFYEWFERQLR (SEQ ID NO:1565);

GFWDESFYAWFERQLR (SEQ ID NO:1566);

GFMDESFYAWFERQLR (SEQ ID NO:1567);

GFWDESFYEWFERQLR (SEQ ID NO:1568);

RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2130); and

S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

12. The method according to claim 2, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of:

WLDQEWWQCEVYGRGCPSKK (SEQ ID NO:2227); KWLDQEWAQVQCEVYGRGCPSKK (SEQ ID NO:1579); KWLDQEWAQVQCEVYGRGCPS (SEQ ID NO:1580);

SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581);

SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582);

SLEEEWAQIECEVYGRGCPS (SEQ ID NO:1583); and

SLEEEWAQIECEVWGRGCPS (SEQ ID NO:1584).

13. The method according to claim 2, wherein the amino acid sequence is selected from the group consisting of sequences 537-538 (SEQ ID NOS:2114-2115).

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14. The method according to claim 2, wherein the amino acid sequence is selected from the group consisting of sequences S425 (SEQ ID NO:2031), S454 (SEQ ID NOS:2058-2059), S459 (SEQ ID NO:2065), and RB537-RB538 (SEQ ID NOS:2197-2198).
15. The method according to claim 1, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .
16. The method according to claim 15, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
17. The method according to claim 15, wherein X_{24} and X_{39} are cysteines, X_{23} is selected from leucine, isoleucine, methionine and valine; X_{27} is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X_{31} is tryptophan, X_{32} is glycine; and X_{36} is any aromatic amino acid.
18. The method according to claim 15, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
19. The method according to claim 15, wherein the Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ is HLCVLEELFWGASLFGYCSG (SEQ ID NO:1576).

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20. The method according to claim 15, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
21. The method according to claim 15, wherein the Formula 4 sequence is selected from the group consisting of sequences SEQ ID NOS:713-925 (Figures 2A-2E); SEQ ID NOS:1254-1261 (Figure 9B); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
22. The method according to claim 15, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO: 1600), S342 (SEQ ID NO: 1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170AA), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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23. The method according to claim 15, wherein the Formula 4 sequence is selected from the group consisting of sequences S262 (SEQ ID NO:1898), S282-S283 (SEQ ID NOS:1907-1908), S331 (SEQ ID NO:1949), RB505M (SEQ ID NO:2160), RB446 (SEQ ID NO:2180), and RB505 (SEQ ID NO:2191).
24. The method according to claim 15, wherein the Formula 4 sequence is selected from the group consisting of sequences RB517M (SEQ ID NO:2161), RB515 (SEQ ID NO:2162), and RB510 (SEQ ID NO:2163).
25. The method according to claim 15, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117:

FHENFYDWFVRQVSKK (SEQ ID NO:1556);

FHENFYDWFVRQVS (SEQ ID NO:1557);

RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);

GSLDESFYDWFERQLG (SEQ ID NO:1559);

GLADEDFYEWFERQLR (SEQ ID NO:1561);

GLADEFYEWFDRQLS (SEQ ID NO:1562);

GQLDEDFYEWFDRQLS (SEQ ID NO:1563);

GQLDEDFYAWFDRQLS (SEQ ID NO:1564);

GFMDESFYEWFERQLR (SEQ ID NO:1565);

GFWDESFYAWFERQLR (SEQ ID NO:1566);

GFMDESFYAWFERQLR (SEQ ID NO:1567);

GFWDESFYEWFERQLR (SEQ ID NO:1568);

RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2130); and

S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

26. The method according to claim 15, wherein the amino acid sequence is selected from the group consisting of sequences 431-433 (SEQ ID NOS:2135-2137).

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27. The method according to claim 15, wherein the amino acid sequence is selected from the group consisting of sequences RB431-RB433 (SEQ ID NOS:2184, 2186 and 2188).
28. A method of increasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence in an amount sufficient to increase insulin activity, wherein the amino acid sequence comprises a subsequence that comprises a sequence that binds to Site 1 of insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin receptor, and wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 2 to Site 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.
29. The method according to claim 28, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
30. The method according to claim 29, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.

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31. The method according to claim 29, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
32. The method according to claim 29, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
33. The method according to claim 29, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCPS (SEQ ID NO:2129).
34. The method according to claim 29, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
35. The method according to claim 29, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NOS:926-1061 (Figures 3A-3E); SEQ ID NOS:1244-1253 (Figure 9A); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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36. The method according to claim 29, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1990-1991), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, 1900-1901), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).
37. The method according to claim 29, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).

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38. The method according to claim 29, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117:

FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2130); and
S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

39. The method according to claim 29, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of:

WLDQEWVQCEVYGRGCPSSK (SEQ ID NO:2227); KWLDQEWAWVQCEVYGRGCPSSK (SEQ ID NO:1579); KWLDQEWAWVQCEVYGRGCPSS (SEQ ID NO:1580);
SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581);
SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582);
SLEEEWAQIECEVYGRGCPSS (SEQ ID NO:1583); and
SLEEEWAQIECEVWGRGCPSS (SEQ ID NO:1584).

40. The method according to claim 29, wherein the amino acid sequence is sequence 539 (SEQ ID NO:2116).

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41. The method according to claim 29, wherein the amino acid sequence is selected from the group consisting of sequences RP27 (SEQ ID NO:2213), RP28 (SEQ ID NO:2214), RP29 (SEQ ID NO:2215), RP30 (SEQ ID NO:2216), RP31 (SEQ ID NO:2217), RP32 (SEQ ID NO:2218), RP33 (SEQ ID NO:2219), RP34 (SEQ ID NO:2220), RP35 (SEQ ID NO:2221), and RP36 (SEQ ID NO:2222).
42. The method according to claim 29, wherein the amino acid sequence is selected from the group consisting of sequences D8-6aa-S175 (SEQ ID NO:2121), D8-12aa-S175 (SEQ ID NO:2122), D8-6aa-RP6 (SEQ ID NO:2126), and D8-6aa-RP17 (SEQ ID NO:2127).
43. The method according to claim 29, wherein the amino acid sequence is selected from the group consisting of sequences S429 (SEQ ID NO:2032), S455 (SEQ ID NO:2060), S457-S458 (SEQ ID NOS:2063-2064), S467-S468 (SEQ ID NOS:2066-2067), S471 (SEQ ID NO:2068), S481-S513 (SEQ ID NOS:2069-2101), S517-S520 (SEQ ID NOS:2104-2107), S524 (SEQ ID NO:2111), RB539 (SEQ ID NO:2196), RB625-RB626 (SEQ ID NOS:2200 and 2199), and RB622 (SEQ ID NO:2201).
44. The method according to claim 28, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .

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45. The method according to claim 44, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
46. The method according to claim 45, wherein X_{24} and X_{39} are cysteines, X_{23} is selected from leucine, isoleucine, methionine and valine; X_{27} is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X_{31} is tryptophan, X_{32} is glycine; and X_{36} is any aromatic amino acid.
47. The method according to claim 45, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
48. The method according to claim 45, wherein the Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33} X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ is HLCVLEELFWGASLFGYCSG (SEQ ID NO:1576).
49. The method according to claim 45, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
50. The method according to claim 45, wherein the Formula 4 sequence is selected from the group consisting of sequences SEQ ID NOS:713-925 (Figures 2A-2E); SEQ ID NOS:1254-1261 (Figure 9B); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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51. The method according to claim 45, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:204).
52. The method according to claim 45, wherein the Formula 4 sequence is selected from the group consisting of sequences S262 (SEQ ID NO:1898), S282-S283 (SEQ ID NOS:1907-1908), S331 (SEQ ID NO:1949), RB505M (SEQ ID NO:2160), RB446 (SEQ ID NO:2180), and RB505 (SEQ ID NO:2191).
53. The method according to claim 45, wherein the Formula 4 sequence is selected from the group consisting of sequences RB517M (SEQ ID NO:2161), RB515 (SEQ ID NO:2162), and RB510 (SEQ ID NO:2163).

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54. The method according to claim 45, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117: FHENFYDWFRQVSKK (SEQ ID NO:1556);
FHENFYDWFRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLAELFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2130); and
S175: GRVDWLQRNANFYDWVVAELG (SEQ ID NO:1560).
55. The method according to claim 45, wherein the amino acid sequence is selected from the group consisting of sequences F8-6aa-RP9 (SEQ ID NO:2119), F8-12aa-RP9 (SEQ ID NO:2120), F8-6aa-S175 (SEQ ID NO:2123), F8-12aa-S175 (SEQ ID NO:2124), S516 (SEQ ID NO:2103), S521 (SEQ ID NO:2108), and S522 (SEQ ID NO:2109).
56. A method of increasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence in an amount sufficient to increase insulin activity, wherein the amino acid sequence comprises a plurality of subsequences that each comprise a sequence that binds to Site 1 of insulin receptor, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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57. The method according to claim 56, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
58. The method according to claim 57, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
59. The method according to claim 57, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
60. The method according to claim 57, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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61. The method according to claim 57, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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62. The method according to claim 57, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117:

FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2130); and
S175: GRVDWLQRNANFYDWVFVAELG (SEQ ID NO:1560).

63. The method according to claim 57, wherein the amino acid sequence is selected from the group consisting of sequences 521 (SEQ ID NO:2112) and 535 (SEQ ID NO:2113).
64. The method according to claim 57, wherein the amino acid sequence is selected from the group consisting of sequences 434 (SEQ ID NO:2138), 436 (SEQ ID NO:2139), 427 (SEQ ID NO:2141), 435 (SEQ ID NO:2142), 439 (SEQ ID NO:2143), 449 (SEQ ID NO:2144), and 463 (SEQ ID NO:2146).

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65. The method according to claim 57, wherein the amino acid sequence is selected from the group consisting of sequences S128 (SEQ ID NOS:1817-1818), S145 (SEQ ID NOS:1822-1823), S169-S170 (SEQ ID NOS:1827-1830), S172 (SEQ ID NOS:1832-1833), S218 (SEQ ID NOS:1850-1851), S228 (SEQ ID NOS:1859-1860), S231-232 (SEQ ID NOS:1863-1866), S253 (SEQ ID NOS:1889-1890), S267 (SEQ ID NO:), S290-S293 (SEQ ID NOS:1914-1921), S300-S301 (SEQ ID NOS:1924-1927), S312 (SEQ ID NOS:1933-1934), S325 (SEQ ID NOS:1944-1945), S329 (SEQ ID NOS:1947-1948), S332-S337 (SEQ ID NOS:1950-1961), S349-S354 (SEQ ID NOS:1965-1976), S359-S363 (SEQ ID NOS:1977-1986), S374-S376 (SEQ ID NOS:1992-1996), S378-S381 (SEQ ID NOS:1997-2004), S414-S418 (SEQ ID NOS:2015-2024), S420 (SEQ ID NOS:2027-2028), RB463 (SEQ ID NO:2165), RB439 (SEQ ID NO:2166), RB436 (SEQ ID NO:2167), RB449 (SEQ ID NO:2168), RB508M-RB509M (SEQ ID NOS:2171-2172), RB508-RB509 (SEQ ID NOS:2189-2190), RB521 (SEQ ID NO:2193), and RB535 (SEQ ID NO:2194).
66. A method of increasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence in an amount sufficient to increase insulin activity, wherein the amino acid sequence comprises a subsequence that comprises a sequence that binds to Site 1 of insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin receptor, wherein the subsequences are linked C-terminus to C-terminus or N-terminus to N-terminus, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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67. The method according to claim 66, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
68. The method according to claim 67, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
69. The method according to claim 67, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
70. The method according to claim 67, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
71. The method according to claim 67, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCP (SEQ ID NO:2056).
72. The method according to claim 67, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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73. The method according to claim 67, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NOS:926-1061 (Figures 3A-3E); SEQ ID NOS:1244-1253 (Figure 9A); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
74. The method according to claim 67, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO: 1600), S342 (SEQ ID NO: 1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).
75. The method according to claim 67, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102) and RB426 (SEQ ID NO:2158).

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76. The method according to claim 67, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117:

FHENFYDWFVRQVSKK (SEQ ID NO:1556);

FHENFYDWFVRQVS (SEQ ID NO:1557);

RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);

GSLDESFYDWFERQLG (SEQ ID NO:1559);

GLADEDFYEWFERQLR (SEQ ID NO:1561);

GLADEFYEWFDRQLS (SEQ ID NO:1562);

GQLDEDFYAWFDRQLS (SEQ ID NO:1563);

GQLDEDFYAWFDRQLS (SEQ ID NO:1564);

GFMDESFYEWFERQLR (SEQ ID NO:1565);

GFWDESFYAWFERQLR (SEQ ID NO:1566);

GFMDESFYAWFERQLR (SEQ ID NO:1567);

GFWDESFYEWFERQLR (SEQ ID NO:1568);

RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and

S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

77. The method according to claim 67, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of:

WLDQE WVQCEVYGRGCPSKK (SEQ ID NO:2227); KWLDQE WAQVQCEVYGRGCPSKK (SEQ ID NO:1579); KWLDQE WAQVQCEVYGRGCPS (SEQ ID NO:1580);

SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581);

SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582);

SLEEEWAQIECEVYGRGCP (SEQ ID NO:1583); and

SLEEEWAQIECEVWGRGCP (SEQ ID NO:1584).

78. The method according to claim 67, wherein the amino acid sequence is selected from the group consisting of sequences S432-S433 (SEQ ID NOS:2033-2036), S436-S445 (SEQ ID NOS:2037-2056), and S456 (SEQ ID NOS:2061-2062).

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79. An amino acid sequence that is an insulin receptor agonist, which comprises a subsequence that comprises a sequence that binds to Site 1 of the insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of the insulin receptor, wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 2 to Site 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.
80. The amino acid sequence according to claim 79, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
81. The amino acid sequence according to claim 80, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
82. The amino acid sequence according to claim 80, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
83. The amino acid sequence according to claim 80, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).

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84. The amino acid sequence according to claim 80, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCPS (SEQ ID NO:2056).
85. The amino acid sequence according to claim 80, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
86. The amino acid sequence according to claim 80, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NOS:926-1061 (Figures 3A-3E); SEQ ID NOS:1244-1253 (Figure 9A); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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87. The amino acid sequence according to claim 80, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).
88. The amino acid sequence according to claim 80, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).

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89. The amino acid sequence according to claim 80, wherein the Formula 1 sequence is selected from the group consisting of sequences H2C/D117:
FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and
S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).
90. The amino acid sequence according to claim 80, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of: WLDQE WVQCEVYGRGCP SKK (SEQ ID NO:2227); KWLDQE WA VQCEVYGRGCP SKK (SEQ ID NO:1579); KWLDQE WA VQCEVYGRGCP S (SEQ ID NO:1580);
SLEEEWAQIQCEIYGRGC RY (SEQ ID NO:1581);
SLEEEWAQIQCEIWGRGC RY (SEQ ID NO:1582);
SLEEEWAQIECEVYGRGCP S (SEQ ID NO:1583); and
SLEEEWAQIECEVWGRGCP S (SEQ ID NO:1584).
91. The amino acid sequence according to claim 80, wherein the amino acid sequence is sequence 539 (SEQ ID NO:2116).

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92. The amino acid sequence according to claim 80, wherein the amino acid sequence is selected from the group consisting of sequences S429 (SEQ ID NO:2032), S455 (SEQ ID NO:2060), S457-S458 (SEQ ID NOS:2063-2064), S467-S468 (SEQ ID NOS:2066-2067), S471 (SEQ ID NO:2068), S471 (SEQ ID NO:2068), S481-S513 (SEQ ID NOS:2069-2101), S517-S520 (SEQ ID NOS:2104-2107), S524(SEQ ID NO:2111), RB539 (SEQ ID NO:2196), RB625-RB626 (SEQ ID NOS:2200 and 2199), and RB622 (SEQ ID NO:2201).
93. The amino acid sequence according to claim 80, wherein the amino acid sequence is selected from the group consisting of sequences RP27 (SEQ ID NO:2213), RP28 (SEQ ID NO:2214), RP29 (SEQ ID NO:2215), RP30 (SEQ ID NO:2216), RP31 (SEQ ID NO:2217), RP32 (SEQ ID NO:2218), RP33 (SEQ ID NO:2219), RP34 (SEQ ID NO:2220), and RP35 (SEQ ID NO:2221).
94. The amino acid sequence according to claim 80, wherein the amino acid sequence is selected from the group consisting of sequences D8-6aa-S175 (SEQ ID NO:2121), D8-12aa-S175 (SEQ ID NO:2122), D8-6aa-RP15 (SEQ ID NO:2126), and D8-6aa-RP17 (SEQ ID NO:2127).
95. The amino acid sequence according to claim 79, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .

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96. The amino acid sequence according to claim 95, wherein X₁, X₂, and X₅ are selected from the group consisting of phenylalanine and tyrosine, X₃ is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X₄ is selected from group consisting of tryptophan, tyrosine and phenylalanine.
97. The amino acid sequence according to claim 95, wherein X₂₄ and X₃₉ are cysteines, X₂₃ is selected from leucine, isoleucine, methionine and valine; X₂₇ is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X₃₁ is tryptophan, X₃₂ is glycine; and X₃₆ is any aromatic amino acid.
98. The amino acid sequence according to claim 95, wherein the Formula 1 sequence X₁X₂X₃X₄X₅ is FYDWF (SEQ ID NO:1554).
99. The amino acid sequence according to claim 95, wherein the Formula 4 sequence X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀ X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈ X₃₉X₄₀X₄₁ is HLCVLEELFWWGASLFGYCSG (SEQ ID NO:1576).
100. The amino acid sequence according to claim 95, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
101. The amino acid sequence according to claim 95, wherein the Formula 4 sequence is selected from the group consisting of sequences S262 (SEQ ID NO:1898), S282-S283 (SEQ ID NOS:1907-1908), S331 (SEQ ID NO:1949), RB505M (SEQ ID NO:2160), RB446 (SEQ ID NO:2180), and RB505 (SEQ ID NO:2191).

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102. The amino acid sequence according to claim 95, wherein the Formula 4 sequence is selected from the group consisting of sequences RB517M (SEQ ID NO:2161), RB515 (SEQ ID NO:2162), and RB510 (SEQ ID NO:2163).
103. The amino acid sequence according to claim 95, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558 and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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104. The amino acid sequence according to claim 95, wherein the Formula 1 sequence is selected from the group consisting of sequences
H2C/D117:
FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: **GSLDESFYDWFERQLGKK (SEQ ID NO:1558);**
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYAWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: **SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057);** and
S175: **GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).**
105. The amino acid sequence according to claim 95, wherein the amino acid sequence is selected from the group consisting of sequences F8-6aa-RP9 (SEQ ID NO:2119), F8-12aa-RP9 (SEQ ID NO:2120), F8-6aa-S175 (SEQ ID NO:2123), F8-12aa-S175 (SEQ ID NO:2124), S516 (SEQ ID NO:2103), S521 (SEQ ID NO:2108), and S522 (SEQ ID NO:2109).
106. An amino acid sequence that is an insulin receptor agonist, which comprises a plurality of subsequences that each comprise a sequence that binds to Site 1 of insulin receptor, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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107. The amino acid sequence according to claim 106, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$, wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
108. The amino acid sequence according to claim 107, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
109. The amino acid sequence according to claim 107, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
110. The amino acid sequence according to claim 107, wherein the Formula 1 sequence is selected from the group consisting of SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NO:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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111. The amino acid sequence according to claim 107, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO: 1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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112. The amino acid sequence according to claim 107, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117: FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and
S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

113. The amino acid sequence according to claim 107, wherein the amino acid sequence is selected from the group consisting of sequences 521 (SEQ ID NO:2112) and 535 (SEQ ID NO:2113).
114. The amino acid sequence according to claim 107, wherein the amino acid sequence is selected from the group consisting of sequences 434 (SEQ ID NO:2138), 436 (SEQ ID NO:2139), 427 (SEQ ID NO:2141), 435 (SEQ ID NO:2142), 439 (SEQ ID NO:2143), 449 (SEQ ID NO:2144), and 463 (SEQ ID NO:2146).

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115. The amino acid sequence according to claim 107, wherein the amino acid sequence is selected from the group consisting of sequences S128 (SEQ ID NOS:1817-1818), S145 (SEQ ID NOS:1822-1823), S169-S170 (SEQ ID NOS:1827-1830), S172 (SEQ ID NOS:1832-1833), S218 (SEQ ID NOS:1850-1851), S228 (SEQ ID NOS:1859-1860), S231-232 (SEQ ID NOS:1863-1866), S253 (SEQ ID NOS:1889-1890), S267 (SEQ ID NO:), S290-S293 (SEQ ID NOS:1914-1921), S300-S301 (SEQ ID NOS:1924-1927), S312 (SEQ ID NOS:1933-1934), S325 (SEQ ID NOS:1944-1945), S329 (SEQ ID NOS:1947-1948), S332-S337 (SEQ ID NOS:1950-1961), S349-S354 (SEQ ID NOS:1965-1976), S359-S363 (SEQ ID NOS:1977-1986), S374-S376 (SEQ ID NOS:1992-1996), S378-S381 (SEQ ID NOS:1997-2004), S414-S418 (SEQ ID NOS:2015-2024), S420 (SEQ ID NOS:2027-2028), RB463 (SEQ ID NO:2165), RB439 (SEQ ID NO:2166), RB436 (SEQ ID NO:2167), RB449 (SEQ ID NO:2168), RB508M-RB509M (SEQ ID NOS:2171-2172), RB508-RB509 (SEQ ID NOS:2189-2190), RB521 (SEQ ID NO:2193), and RB535 (SEQ ID NO:2194).
116. An amino acid sequence that is an insulin receptor agonist, which comprises a subsequence that comprises a sequence that binds to Site 1 of insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin receptor, wherein the subsequences are linked C-terminus to C-terminus or N-terminus to N-terminus, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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117. The amino acid sequence according to claim 116, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
118. The amino acid sequence according to claim 117, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
119. The amino acid sequence according to claim 117, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
120. The amino acid sequence according to claim 117, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
121. The amino acid sequence according to claim 117, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCP (SEQ ID NO:2056).

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122. The amino acid sequence according to claim 117, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NO:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
123. The amino acid sequence according to claim 117, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NOS:926-1061 (Figures 3A-3E); SEQ ID NOS:1244-1253 (Figure 9A); and SEQ ID NO:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
124. The amino acid sequence according to claim 117, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO: 1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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125. The amino acid sequence according to claim 117, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NO:DD-1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).
126. The amino acid sequence according to claim 117, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117: FHENFYDWFRQVSKK (SEQ ID NO:1556);
FHENFYDWFRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and
S175: GRVDWLQRNANFYDWVAELG (SEQ ID NO:1560).

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127. The amino acid sequence according to claim 117, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of:

WLDQEWWQCEVYGRGCPSSK (SEQ ID NO:2227);
KWLDQEWAQVQCEVYGRGCPSSK (SEQ ID NO:1579);
KWLDQEWAQVQCEVYGRGCPSS (SEQ ID NO:1580);
SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581);
SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582);
SLEEEWAQIECEVYGRGCPSS (SEQ ID NO:1583); and
SLEEEWAQIECEVWGRGCPSS (SEQ ID NO:1584).

128. The amino acid sequence according to claim 117, wherein the amino acid sequence is selected from the group consisting of sequences S432-S433 (SEQ ID NOS:2033-2036), S436-S445 (SEQ ID NOS:2037-2056), and S456 (SEQ ID NOS:2061-2062).
129. An amino acid sequence that is an insulin receptor antagonist, which comprises a subsequence that comprises a sequence that binds to Site 1 of insulin receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin receptor, wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 1 to Site 2, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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130. The amino acid sequence according to claim 129, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
131. The amino acid sequence according to claim 130, wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
132. The amino acid sequence according to claim 130, wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
133. The amino acid sequence according to claim 130, wherein the Formula 1 sequence $X_1X_2X_3X_4X_5$ is FYDWF (SEQ ID NO:1554).
134. The amino acid sequence according to claim 130, wherein the Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ is WLDQEWAQVQCEVYGRGCPs (SEQ ID NO:2056).

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135. The amino acid sequence according to claim 130, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NO:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
136. The amino acid sequence according to claim 130, wherein the Formula 6 sequence is selected from the group consisting of sequences SEQ ID NO:926-1061 (Figures 3A-3E); SEQ ID NO:1244-1253 (Figure 9A); and SEQ ID NO:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
137. The amino acid sequence according to claim 130, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251 (SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NOS:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

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138. The amino acid sequence according to claim 130, wherein the Formula 6 sequence is selected from the group consisting of sequences S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).
139. The amino acid sequence according to claim 130, wherein the Formula 1 sequence is selected from the group consisting of sequences
H2C/D117: FHENFYDWFVRQVSKK (SEQ ID NO:1556);
FHENFYDWFVRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADELFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYEWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and
S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

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140. The amino acid sequence according to claim 130, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of:

WLDQEWWQCEVYGRGCP SKK (SEQ ID NO:2227);
KWLDQEWWQCEVYGRGCP SKK (SEQ ID NO:1579);
KWLDQEWWQCEVYGRGCP S (SEQ ID NO:1580);
SLEEEWAQIQCEIYGRGC RY (SEQ ID NO:1581);
SLEEEWAQIQCEIWGRGC RY (SEQ ID NO:1582);
SLEEEWAQIECEVYGRGCP S (SEQ ID NO:1583); and
SLEEEWAQIECEVWGRGCP S (SEQ ID NO:1584).

141. The amino acid sequence according to claim 130, wherein the amino acid sequence is selected from the group consisting of sequences 537-538 (SEQ ID NOS:2114-2115).
142. The amino acid sequence according to claim 130, wherein the amino acid sequence is selected from the group consisting of sequences S425 (SEQ ID NO:2031), S454 (SEQ ID NOS:2058-2059), S459 (SEQ ID NO:2065), and RB537-RB538 (SEQ ID NOS:2197-2198).
143. The amino acid sequence according to claim 129, wherein the Site 1 sequences consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 4 sequence $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .

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144. The amino acid sequence according to claim 143, wherein X₁, X₂, and X₅ are selected from the group consisting of phenylalanine and tyrosine, X₃ is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X₄ is selected from group consisting of tryptophan, tyrosine and phenylalanine.
145. The amino acid sequence according to claim 143, wherein X₂₄ and X₃₉ are cysteines, X₂₃ is selected from leucine, isoleucine, methionine and valine; X₂₇ is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X₃₁ is tryptophan, X₃₂ is glycine; and X₃₆ is any aromatic amino acid.
146. The amino acid sequence according to claim 143, wherein the Formula 1 sequence X₁X₂X₃X₄X₅ is FYDWF (SEQ ID NO:1554).
147. The amino acid sequence according to claim 143, wherein the Formula 4 sequence X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁ is HLCVLEELFWGASLFGYCSG (SEQ ID NO:1576).
148. The amino acid sequence according to claim 143, wherein the Formula 1 sequence is selected from the group consisting of sequences SEQ ID NOS:1-712 (Figures 1A-1O); SEQ ID NOS:1221-1243 (Figure 8); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).
149. The amino acid sequence according to claim 143, wherein the Formula 4 sequence is selected from the group consisting of sequences SEQ ID NOS:713-925 (Figures 2A-2E); SEQ ID NOS:1254-1261 (Figure 9B); and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776 (Table 2).

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150. The amino acid sequence according to claim 143, wherein the Formula 1 sequence is selected from the group consisting of sequences S105-S116 (SEQ ID NOS:1791-1805), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1900-1901), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, and 1990-1991), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).
151. The amino acid sequence according to claim 143, wherein the Formula 4 sequence is selected from the group consisting of sequences S262 (SEQ ID NO:1898), S282-S283 (SEQ ID NOS:1907-1908), S331 (SEQ ID NO:1949), RB505M (SEQ ID NO:2160), RB446 (SEQ ID NO:2180), and RB505 (SEQ ID NO:2191).
152. The amino acid sequence according to claim 143, wherein the Formula 4 sequence is selected from the group consisting of sequences RB517M (SEQ ID NO:2161), RB515 (SEQ ID NO:2162), and RB510 (SEQ ID NO:2163).

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153. The amino acid sequence according to claim 143, wherein the Formula 1 sequence is selected from the group consisting of sequences

H2C/D117: FHENFYDWFRQVSKK (SEQ ID NO:1556);
FHENFYDWFRQVS (SEQ ID NO:1557);
RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558);
GSLDESFYDWFERQLG (SEQ ID NO:1559);
GLADEDFYEWFERQLR (SEQ ID NO:1561);
GLADEFYEWFDRQLS (SEQ ID NO:1562);
GQLDEDFYAWFDRQLS (SEQ ID NO:1563);
GQLDEDFYAWFDRQLS (SEQ ID NO:1564);
GFMDESFYEWFERQLR (SEQ ID NO:1565);
GFWDESFYAWFERQLR (SEQ ID NO:1566);
GFMDESFYAWFERQLR (SEQ ID NO:1567);
GFWDESFYEWFERQLR (SEQ ID NO:1568);
RP15: SQAGSAFYAWFDQVLRTV (SEQ ID NO:2057); and
S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560).

154. The amino acid sequence according to claim 143, wherein the amino acid sequence is selected from the group consisting of sequences 431-433 (SEQ ID NOS:2135-2137).
155. The amino acid sequence according to claim 143, wherein the amino acid sequence is selected from the group consisting of sequences RB431-RB433 (SEQ ID NOS:2184, 2186, and 2188).
156. A pharmaceutical composition comprising the amino acid sequence according to claim 79, and a physiologically acceptable carrier, excipient or diluent.
157. A pharmaceutical composition comprising the amino acid sequence according to claim 106, and a physiologically acceptable carrier, excipient or diluent.

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158. A pharmaceutical composition comprising an amino acid sequence according to claim 116, a physiologically acceptable carrier, excipient or diluent.
159. A pharmaceutical composition comprising the amino acid sequence according to claim 129, and a physiologically acceptable carrier, excipient or diluent.
160. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of the pharmaceutical composition according to claim 156, thereby treating the diabetes.
161. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of the pharmaceutical composition according to claim 157, thereby treating the diabetes.
162. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of the pharmaceutical composition according to claim 158, thereby treating the diabetes.
163. A method of treating insulin shock comprising administering to an individual in need of treatment a therapeutically effective amount of the pharmaceutical composition according to claim 159, thereby treating the insulin shock.
164. A method of identifying an insulin agonist comprising:
 - 1) producing a secondary library comprising peptides derived from the amino acid sequence according to claim 79;
 - 2) screening the library peptides for binding to insulin receptor; and
 - 3) screening the insulin-binding peptides from (2) for agonist activity for insulin receptor, wherein agonist activity indicates identification of an insulin receptor agonist.

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165. A method of identifying an insulin agonist comprising:
 - 1) producing a secondary library comprising peptides derived from the amino acid sequence according to claim 106;
 - 2) screening the library peptides for binding to insulin receptor; and
 - 3) screening the insulin-binding peptides from (2) for agonist activity for insulin receptor, wherein agonist activity indicates identification of an insulin receptor agonist.
166. A method of identifying an insulin agonist comprising:
 - 1) producing a secondary library comprising peptides derived from the amino acid sequence according to claim 116;
 - 2) screening the library peptides for binding to insulin receptor; and
 - 3) screening the insulin-binding peptides from (2) for agonist activity for insulin receptor, wherein agonist activity indicates identification of an insulin receptor agonist.
167. A method of identifying an insulin antagonist comprising:
 - 1) producing a secondary library comprising peptides derived from the amino acid sequence according to claim 129;
 - 2) screening the library peptides for binding to insulin receptor; and
 - 3) screening the insulin-binding peptides from (2) for antagonist activity for insulin receptor, wherein antagonist activity indicates identification of an insulin receptor antagonist.
168. A method of increasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence comprising a sequence selected from the group consisting of SLEEEWAQVECEVYGRGCPGSLDESFYDWFERQLG (S519; SEQ ID NO:2033) and SIEEEWAQIKCDVWGRGCP PGLLDESFYHWFDRQLR (S520; SEQ ID NO:2034), and a physiologically acceptable carrier, excipient, or diluent, in amount sufficient to increase insulin activity.

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169. An amino acid sequence that is an insulin receptor agonist, which comprises a sequence selected from the group consisting of SLEEEWAQVECEVYGRGCPGSLDESFYDW FERQLG (S519; SEQ ID NO:2033) and SIEEEWAQIKCDVWGRGCPPGLLDESFYHWFDRQLR (S520; SEQ ID NO:2034).
170. A pharmaceutical composition comprising the amino acid sequence according to claim 169, and a physiologically acceptable carrier, excipient or diluent.
171. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of the pharmaceutical composition according to claim 170, thereby treating the diabetes.
172. A method of identifying an insulin agonist comprising:
- 1) producing a secondary library comprising peptides derived from the amino acid sequence according to claim 169;
 - 2) screening the library peptides for binding to insulin receptor; and
 - 3) screening the insulin-binding peptides from (2) for agonist activity for insulin receptor, wherein agonist activity indicates identification of an insulin receptor agonist.

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173. A method of increasing insulin-like growth factor receptor activity in mammalian cells comprising administering to said cells an amino acid sequence in an amount sufficient to increase insulin-like growth factor activity, wherein the amino acid sequence comprises a subsequence that comprises a sequence that binds to Site 1 of insulin-like growth factor receptor and a subsequence that comprises a sequence that binds to Site 2 of insulin-like growth factor receptor, and wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 2 to Site 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.
174. The method according to claim 173, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
175. An amino acid sequence that is an insulin-like growth factor receptor agonist, which comprises a subsequence that comprises a sequence that binds to Site 1 of the insulin-like growth factor receptor and a subsequence that comprises a sequence that binds to Site 2 of the insulin-like growth factor receptor, wherein the subsequences are linked C-terminus to N-terminus and the subsequences are oriented Site 2 to Site 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.

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176. The amino acid sequence of claim 175, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$ and the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, and wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid, and wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.
177. An amino acid sequence that binds to insulin-like growth factor receptor, which comprises a sequence selected from the group consisting of a sequence that binds to Site 1 of the insulin-like growth factor receptor and a sequence that binds to Site 2 of the insulin-like growth factor receptor, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, or fragments thereof.
178. The amino acid sequence of claim 177, wherein the Site 1 sequence consists essentially of a Formula 1 sequence $X_1X_2X_3X_4X_5$, wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
179. The amino acid sequence of claim 177, wherein the Site 1 sequence consists essentially of a Formula 2 sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids.
180. The amino acid sequence of claim 177, wherein the Site 2 sequence consists essentially of a Formula 6 sequence $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, wherein X_{62} , X_{65} , $X_{66} X_{68}$, X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{79} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

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181. The method according to claim 29, wherein the amino acid sequence is selected from the group consisting of: S527-S546; S549, D551-S591; S594-S624; S626-S639; and S641-S648.
182. The method according to claim 181, wherein the amino acid sequence is selected from the group consisting of S557 and S597.
183. A method of increasing insulin receptor activity in mammalian cells comprising administering to said cells an amino acid sequence selected from the group consisting of: S527-S546; S549, D551-S591; S594-S624; S626-S639; and S641-S648 and a physiologically acceptable carrier, excipient, or diluent, in an amount sufficient to increase insulin receptor activity.
184. The method according to claim 183, wherein the amino acid sequence is selected from the group consisting of S557 and S597.
185. An amino acid sequence that is an insulin receptor agonist, which comprises a sequence selected from the group consisting of: S527-S546; S549, D551-S591; S594-S624; S626-S639; and S641-S648.
186. The sequence according to claim 185, wherein the sequence is selected from the group consisting of S557 and S597.
187. A method of treating diabetes, said method comprising administering to a patient in need of such treatment (i) a first amount of a first compound selected from the group consisting of: peptides S519, S520; S524, S527-S546, S549, D551-S591, S594-S624, S626-S639, and S641-S648 and (ii) a second amount of a second compound comprising a long-acting insulin analogue, wherein the first and second amounts together are effective for treating said diabetes.
188. The method according to claim 187, wherein said first compound is selected from the group consisting of: S557 and S597.

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189. The method according to claim 187, wherein said long-acting insulin analogue is selected from the group consisting of LysB29(- myristoyl)des(B30) human insulin, LysB29(-tetradecanoyl)des(B30) human insulin, and B29-N -(N-lithocolyl- -glutamyl)-des(B30) human insulin

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR	
R40-3-40B2-IR	XXXXXXXXXXXXXX	--	--	--	--	--	
R40-4-40B12-IR	IRDMHYYWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID	40.3	9.0	2.0	4.5	0.2	
R40-4-40G11-IR	RMGLQ ^Q ALAHYRKSAAGPIFLSSSGSVIKGSEGDPFYAWFRLQ	60.4	12.9	2.0	6.5	0.2	
	MPVSLI ^F RRVWDDYRDGEHETLESHYVV ^P QAA ^L DKL ^K FYSWFS	52.6	37.5	2.0	18.8	0.1	

FIG. 1A

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR	
R40-3-D5-IGFR	XXXXXXXXXXXXXX	--	--	--	--	--	
R40-3-A6-IGFR	PLYGGGIHLYYPGTMGYVPGFPQRQVKVLGDA ^D AKNFYDWFM	--	--	--	--	--	
R40-X-R35-IGFR	YRGMLVLGRISDGAGKVASEPPARIQKVFAVNFYDWFV	--	--	--	--	--	
	SGCCRLLGLRWMFIVVGWSGALVCQSAAASAAAGFYDWFV	--	--	--	--	--	

FIG. 1B

Clone Design	Sequence	Comparisons			
		E-Tag	IGFsR	IR	IR/IGFR
Ratios over Background					
R20 α -3-20D3-IR	XXXXXXXXXXXXXX	46.3	36.2	7.0	5.2
R20 α -3-20F1-IR	IGGQQQHQDGDNFYDWFWVEALA	49.0	26.0	2.8	9.3
R20 α -3-20H1-IR	VFWNCRSQQQLDFYEWFE <u>QAA</u>	45.6	35.3	3.3	10.7
R20 α -3-20D1-IR	RVAGAIISAPGLVSNKQDGFLFYSWFRE	50.8	37.5	3.0	12.5
R20 β -4-B12-IR	VI <u>Q</u> ARHGCDSVSDCFYEWFA	41.9	2.9	5.7	0.5
R20 β -4-H3-IR	GAFYRWFH EALVGS ERVPDV	13.9	5.8	2.4	0.4
R20 β -4-D10-2-IR	HEAFYDWFSALVDGGYELMG	21.5	7.3	2.9	2.5
R20 β -4-C8-IR	RIGGGWARSEG FYEW FVREL	44.9	31.1	9.6	3.2
R20 β -4-E7-IR	LPGAGA?GFA?RGFYEW FES	45.0	18.8	5.9	3.2
R20 β -4-E7-2-IR	GHSWALVRHVDRFLFYEW FDL	45.0	18.8	5.9	3.2
R20 β -4-G3-IR	LGTSAQGVGVHRAFYQW FQS	38.6	7.5	2.0	3.8
R20 β -4-H6-IR	RGGGTFYEW FES ALRKHGAG	14.8	7.6	2.0	3.8
R20 β -4-G11-IR	NSSGQQVVG LTF YSWFASQV	39.4	7.5	1.9	3.9
R20 β -4-H8-IR	FYGWFSRQLSLLPRDDWGLP	41.2	15.1	3.4	4.4
R20 β -4-H9-IR	RMFYEW FW SQMGAGPTEGSA	43.1	8.8	2.0	4.4
R20 β -4-H8-IR	IGGQQHQDGNFYDWFWVEALA	47.9	43.7	9.3	4.7
R20 β -4-B8-IR	RDKPTDQEEQNWSFYEW ERH	16.3	13.9	2.4	5.8
R20 β -4-E2-IR	WSALLSVM DTGF YAWFDDAV	15.3	5.9	1.0	5.9
R20 β -4-F4-IR	SRDQTNFTNSAGFYGW FER	44.0	40.1	8.4	4.8
R20 β -4-A8-IR	GVGTLTMSSDAFYTW EV	17.3	44.8	36.0	6.0
R20 β -4-C4-IR	IGGSFVEFYGW NDQV	44.8	36.2	17.3	4.3
R20 β -4-D7-IR	DIGSDGHGRWIDSFYRW TEM	31.2	29.4	4.3	6.2
R20 β -4-D2-IR	VLQARHGCDSVSDCFYEWFA	31.2	29.4	5.6	6.5
	DPERMQSDVGFYEW FR AVG				0.1

FIG. 1C

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF1R	IR	IGFR/IR	IR/IGFR	
R20-4-B9-IGFR	XXXXXXXXXXXXXX	--	--	--	--	--	
R20-4-F8-IGFR	DPERMQS DVGFYEW FRAAVG	40.1	16.6	--	--	--	
R20-4-G12-IGFR	DIGSDGHGR RWDSFYRW FEM	39.2	13.9	--	--	--	
R20-4-D10-IGFR	PFYQWFLD QSVGG SRG GGLR	36.7	8.0	--	--	--	
	AVAPLSVVRGR DSGFY SWFSS	40.2	4.1	--	--	--	

FIG. 1D

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Clone Design	Sequence	Comparisons					
		Background E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
A6S-3-E12-IR	XXXXXXXXXNFXFYDWFFVXXXX	26.2	1.3	8.0	0.2	6.2	
A6S-2-C1-IR	GRVDWLQRNANFNFYDWFFVAELG	41.2	1.3	7.0	0.2	5.4	
A6S-1-A7-IR	RMYFSTGAPQNQNFYDWFFVQEWD	47.2	2.3	11.1	0.2	4.8	
A6S-2-C8-IR	HHTQGLQVQRNFNFYDWFFVNELL	44.9	1.5	5.5	0.3	3.7	
A6S-3-E10-IR	MHRMQHDGTSNENFNFYDWFFVLQWA	46.9	1.6	5.0	0.3	3.1	
A6S-2-D5-IR	AMHVVAQGGPNFNFYDWFFVRELR	31.9	1.2	3.7	0.3	3.1	
A6S-1-B2-IR	AIQMNGNLAFNFNFYDWFFVRELT	31.6	1.8	5.3	0.3	2.9	
A6S-1-A4-IR	TDRKSVQEPRNFNFYDWFFVWAAR	43.3	3.6	9.2	0.4	2.6	
A6S-4-G3-IR	PHGHRGFAQSNENFNFYDWFFVQEE	31.3	2.3	5.1	0.5	2.2	
A6S-4-H8-IR	RLASASVPGQNFNFYDWFFVQDQLL	11.5	1.7	3.6	0.5	2.1	
A6S-3-E11-IR	RQSEFSTLNENFNFYDWFFVRELE	26.3	2.3	4.4	0.5	1.9	
A6S-1-A1-IR	GQAQLSIRDVNFNFYDWFFVQQLV	36.9	3.7	6.5	0.6	1.8	
A6S-2-C9-IR	MSEPAVGVNNGNFNFYDWFFVQQLF	43.6	1.3	2.3	0.6	1.8	
A6S-2-C4-IR	VGTGRARLDRNFNFYDWFFVQYS	34.5	5.6	9.6	0.6	1.7	
A6S-4-H10-IR	SREAQKRNNANFNFYDWFFVQLS	39.2	4.4	6.9	0.6	1.6	
A6S-4-G7-IR	LAQFAGSRNRNQNFNFYDWFFVQLG	19.1	1.4	2.2	0.6	1.6	
A6S-4-H2-IR	GQEYFDQMGHLNFNFYDWFFVRELD	25.5	2.6	3.9	0.7	1.5	
A6S-2-C3-IR	RQPSQQPHGSNFNFYDWFFVEAIN	31.1	1.6	2.4	0.7	1.5	
A6S-2-C11-IR	IMQSLLGSGSTNFNFYDWFFQQMV	20.9	3.3	4.6	0.7	1.4	
A6S-3-F3-IR	DQQRSACDGTNENFNFYDWFFVCQLS	37.1	3.0	4.2	0.7	1.4	
A6S-3-E5-IR	LDGTKACQRVNFNFYDWFFVCQTE	31.6	2.5	3.5	0.7	1.4	
A6S-1-B7-IR	PEARRTVVHSNFNFYDWFFVAQLS	49.2	1.6	2.3	0.7	1.4	
A6S-3-E7-IR	PWMLSVGIQDNNFNFYDWFFVGLDS	37.2	5.0	6.3	0.8	1.3	
A6S-4-G6-IR	ASHQRGGSSDNNFNFYDWFFVAQMR	16.8	3.1	4.0	0.8	1.3	
A6S-2-C2-IR	TLEREGEFGSGNFNFYDWFFVEQLH	29.7	2.4	3.1	0.8	1.3	
A6S-3-F1-IR	DRQSIGSVHGDFFYDWFFVSALG	29.7	2.3	3.0	0.8	1.3	
A6S-2-C5-IR	DWDKLGSLSSENENFNFYDWFFVDQLA	42.9	6.1	7.0	0.9	1.1	
A6S-3-E4-IR	VRVVLNQSGRNNFNFYDWFFVIQLE	20.9	2.1	2.3	0.9	1.1	
	MASWQSRTPDNFNFYDWFFVRELS						

FIG. 1E-1

Clone Design	Sequence	Comparisons				5/122
		IGFR/IR	IR	IGF ₁ R	IGFR/IR	
A6S-3-E9-IR	XXXXXXXXXXXXXXNFXDWFWVXXXXX	36.6	9.0	8.9	1.0	1.0
A6S-3-E1-IR	TTCHPRGEDCNFYDWFWVQLR	36.7	6.8	6.9	1.0	1.0
A6S-4-H12-IR	VRGNDSVLRLANFYDWFWVQQLS	46.3	6.1	5.8	1.1	1.0
A6S-2-D3-IR	TPRSQVRSDHNNFYDWFWVYQLA	37.0	5.3	5.1	1.0	1.0
A6S-3-E8-IR	ESLTGSRPDRNMFYDWFWVQQTS	42.7	5.2	5.1	1.0	1.0
A6S-1-A12-IR	POSLTEVRTGNMFYDWFWVQLH	39.7	2.1	2.1	1.0	1.0
A6S-4-H3-IR	DVGMRVKE TNFYDWFWVQLI	18.6	3.1	2.9	1.1	0.9
A6S-3-F7-IR	GADDIIRSLNTNFYDWFWVNQLS	46.2	2.3	2.1	1.1	0.9
A6S-2-D8-IR	GVS1QAGYKTNMFYDWFWVEAVR	31.2	2.0	1.7	1.2	0.9
A6S-3-F10-IR	VGEHRQMSVGNMFYDWFWVMQIA	39.0	5.9	4.5	1.3	0.8
A6S-4-G11-IR	GSSLGRSGPGNFYDWFWVDQLE	44.8	4.3	3.3	1.3	0.8
A6S-2-D2-IR	HRQQDGVVRQGNFYDWFWVQALE	33.5	3.6	2.7	1.3	0.8
A6S-4-G8-IR	QDTFLTAREGNFYDWFI RALE	11.1	2.5	1.9	1.3	0.8
A6S-4-H6-IR	EAIMREEGQANFYDWFWVRLQE	22.4	2.4	1.9	1.3	0.8
A6S-2-D10-IR	VCDVSTGGGTNFYDWFWVCQVG	41.3	2.1	1.7	1.2	0.8
A6S-3-F4-IR	POPRSASTPLNFYDWFWVQATG	37.0	13.5	9.9	1.4	0.7
A6S-4-G9-IR	GVSRGSGGDPNFYDWFWFMQLR	36.2	11.8	7.8	1.5	0.7
A6S-3-F5-IR	GPPRHDSRGNFYDWFWVQQLA	48.1	7.2	4.8	1.5	0.7
A6S-4-H1-IR	ERFALEVQGSNSNFYDWFWVQVI	18.3	3.6	2.6	1.4	0.7
A6S-3-F6-IR	NLKSSATVGGNFYDWFWVEQL	18.7	2.9	1.9	1.5	0.7
A6S-3-F11-IR	MEGPAGGPLNFYDWFWVAQVD	33.8	2.0	1.4	1.4	0.7
A6S-2-C6-IR	RLDVAGHRRGGNFYDWFWVKQLH	46.7	19.2	12.1	1.6	0.6
A6S-4-G4-IR	PWSDHEALNQNMFYDWFWVSQVL	36.9	18.2	10.7	1.7	0.6
A6S-4-G12-IR	EDRLGNGESTNMFYDWFWVQLA	32.8	12.8	7.9	1.6	0.6
A6S-2-D7-IR	GKLVASTLDDNMFYDWFWVRLQS	33.2	12.0	7.1	1.7	0.6
A6S-4-G10-IR	SGPVVQTQGNMFYDWFWVHQLR	33.9	10.8	6.8	1.6	0.6
A6S-3-F9-IR	VDRAGPAGSDNMFYDWFWVQQLD	44.3	9.6	5.7	1.7	0.6
A6S-3-F2-IR	SLGRNDRPDENFYDWFWVSQVQ	23.2	4.3	2.5	1.7	0.6
	RVMATANAPMNMFYDWFWVQLQ					

FIG. 1E-2

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Clone Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR		
A6S-4-G1-IR	XXXXXXXXXXDGNFYDWFTVXXXX	--	--	--	--	--	--	--
A6S-1-A3-IR	NGVERAGTGDNFYDWFTVAQLH	36.2	31.8	15.7	2.0	0.5		
A6S-3-F12-IR	PFAGKGDKTGNFYDWFTVSITG	39.9	12.6	6.0	2.1	0.5		
A6S-4-G2-IR	GMPOEIMDOVNFYDWFTVAQVD	41.4	7.4	4.0	1.9	0.5		
A6S-1-B1-IR	MGTPAVGDGANFYDWFTVRQLG	26.7	7.0	3.5	2.0	0.5		
A6S-2-D11-IR	SKCKAWYGANNNFYDWFTWQVD	30.6	3.7	1.9	1.9	0.5		
A6S-2-E2-IR	EAASLGSQDRNFYDWFTVRQVV	48.4	37.4	13.5	2.8	0.4		
	VERSASSQDGDNFYDWFTVQIR	37.8	30.6	12.0	2.6	0.4		
	TSEVQRRSQDNFYDWFTVAQVA	33.1	24.7	9.8	2.5	0.4		

FIG. 1E-3

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Clone Design	Sequence	Comparisons			
		E-Tag	Ratios over Background	IGFR/R	IR
A6S-4-E4-IGFR	XXXXXXXXXXNFYDWFWXXXXX	--	--	--	--
A6S-2-D2-IGFR	ERSAAGFREGMNFYDWFWAQN	27	32	--	--
A6S-2-F2-IGFR	RAERGSMRDSNFXDWFVQLP	36	30	--	--
A6S-4-F3-IGFR	LAMSASRPANFYDWFWAQIV	35	30	--	--
A6S-4-G4-IGFR	HNSSSPMRTGNFYDWFWQELR	26	30	--	--
A6S-4-G3-IGFR	SALSGPVQINFYDWFWTGM	26	30	--	--
A6S-2-H2-IGFR	GAQAIREIHHNFYDWFWAQVT	21	29	--	--
A6S-2-E3-IGFR	RGQRESDSGTNMFYDWFWGAIR	40	28	--	--
A6S-4-C6-IGFR	VQEGLSGMEGNNFYDWFWDQLF	36	28	--	--
A6S-4-F5-IGFR	RIDRSSTSFGVNMFYDWFWAQVG	25	28	--	--
A6S-4-H3-IGFR	GSOHSGREPHNFYDWFWAQVG	24	28	--	--
A6S-4-H4-IGFR	GRGDQRHETTNFYDWFWRELQ	20	28	--	--
A6S-2-H1-IGFR	PRMVEKPKSEDNFYDWFTQLS	20	28	--	--
A6S-4-E6-IGFR	RVGIQVDPHTNMFYDWFWIQLT	42	27	--	--
A6S-4-B6-IGFR	RSSGGQLLSQGNNFYDWFWSQL	24	26	--	--
A6S-4-D2-IGFR	SDARQAGLQENFYDWFWSQVR	23	26	--	--
A6S-4-G5-IGFR	PPYRSSRLGENFYDWFMQVR	19	26	--	--
A6S-2-A3-IGFR	QEVTTRDDKNFYDWFWVSQIF	18	26	--	--
A6S-4-E2-IGFR	SRAFYGSTAGNMFYDWFWQAVS	37	25	--	--
A6S-4-G6-IGFR	?DGGSVSSSKGNFYDWFWQQMT	25	25	--	--
A6S-4-D6-IGFR	RLMGGIAEPQNFYDWFWREVA	20	25	--	--
A6S-4-H5-IGFR	SAGHHMPRESNFYDWFWDQVV	25	24	--	--
A6S-4-F4-IGFR	LGAETWDGINNFYDWFWKQVS	22	24	--	--
A6S-4-C3-IGFR	VGHSGVPPYPNMFYDWFTMQVS	22	24	--	--
A6S-4-H6-IGFR	VTMIDKGAAQDNFYDWFWREVA	21	24	--	--
A6S-4-F6-IGFR	HHSPGNEHGYNFYDWFLQVA	19	24	--	--
A6S-3-H1-IGFR	GSIAQLIMRANFYDWFWEQTN	18	24	--	--
	LKGSSQPLSVNMFYDWFWQQIK	17	24	--	--
	PASNKNSLAENFYDWFWQQTR	30	23	--	--

FIG. 1F-1

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Ratios over Background Clone Design	Sequence	Comparisons				
		E-Tag	IGF3R	IR	IGFR/IR	IGFR
A6S-4-A6-IGFR	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-4-E3-IGFR	HVEHMAVGDGNNFYDWFVQLR	21	23	--	--	--
A6S-4-D3-IGFR	RGMGTGMVGRGNFYDWFVGQLR	21	23	--	--	--
A6S-3-E10-IGFR	GLRSEQGNRINNFYDWFVQA <u>I</u>	20	23	--	--	--
A6S-4-D1-IGFR	RVREKLPRPENFYDWFVNQ <u>I</u>	23	22	--	--	--
A6S-4-B2-IGFR	SNPSRQDASVNFYDWFVREVA <u>Q</u>	22	22	--	--	--
A6S-4-A2-IGFR	QSVDLSRPDSNMFYDWFVFEVL <u>S</u>	21	22	--	--	--
A6S-4-A5-IGFR	IGGGQQHQDGNNFYDWFVFEAL <u>A</u>	20	22	--	--	--
A6S-4-C1-IGFR	VEVQRH <u>I</u> RKDNFXYDWFVFKQID	19	22	--	--	--
A6S-4-B1-IGFR	CWARPCGDAANMFYDWFVQQ <u>QAS</u>	16	22	--	--	--
A6S-4-B4-IGFR	RHERGKEGPGNFYDWFVFSQVV	19	21	--	--	--
A6S-4-D4-IGFR	ERSPRPALASNMFYDWFVQQVV	19	21	--	--	--
A6S-3-F8-IGFR	IARMRET <u>F</u> QPNNFYDWFVFDQLA	18	21	--	--	--
A6S-3-H9-IGFR	GRGQQ <u>G</u> LKRKPDNFNYDWFVAA <u>AK</u>	25	20	--	--	--
A6S-3-G2-IGFR	YSIEV <u>Q</u> DWNENMFYDWFVSQLG	23	20	--	--	--
A6S-4-H2-IGFR	TWWMEERKQDNFNYDWFVFGQLK	21	20	--	--	--
A6S-4-A3-IGFR	VTFTSAVFHENFYDWFVFRQVS	19	20	--	--	--
A6S-3-G10-IGFR	LAINDLVTHKNFYDWFVFDQLR	18	20	--	--	--
A6S-3-E5-IGFR	GAVGLAEAGPNFYDWFVFSQV <u>Q</u>	24	19	--	--	--
A6S-3-H2-IGFR	RYRGERHDGRNFXDWFVEQVN <u>Q</u>	21	19	--	--	--
A6S-3-G3-IGFR	QGAEGRLSEGNNFYDWFVQAVS	21	19	--	--	--
A6S-4-H1-IGFR	PRLHMGSMDMGDFYDWFVQIA	21	19	--	--	--
A6S-4-G1-IGFR	IVAGARHSEVNFYDWFVIQVR	18	18	--	--	--
A6S-4-A1-IGFR	AELVGAGVRGNNFYDWFVDQLV	16	16	--	--	--
A6S-2-F1-IGFR	DSSRLWLGERNMFYDWFVQA <u>I</u>	17	12	--	--	--
A6S-2-G1-IGFR	VGQVGRYVRSNMFYDWFVQ <u>QAM</u>	30	8	--	--	--
A6S-1-C5-IGFR	RFQLVESGSKMFYDWFVQ <u>QVR</u>	27	7	--	--	--
A6S-2-B2-IGFR	RIHNQTERGGNNFYDWFVH <u>QLV</u>	30	5	--	--	--
	EMYGDTSERVNMFYDWFVSA <u>Q</u>					

FIG. 1F-2

Clone Design	Ratios over Background	Sequence	Comparisons	E-Tag	IGFrR	IR	IGFrR/IR	R/IGFR
A6S-1-D5-IGFR	XXXXXXOOXXXXXNFYDWFFVXOOK	RVSGGMEDLGNFYDWFFVRQAQ	--	--	--	--	--	--
A6S-1-A2-IGFR		KDPVTVSQGRNFYDWFFVVQIQ	2.5	5	--	--	--	--
A6S-3-E6-IGFR		DARDHGWWTMSNMFYDWFFVAQVS	2.0	5	--	--	--	--
A6S-1-G3-IGFR		VATVHVGGMNFYDWFFVAQVG	2.0	5	--	--	--	--
A6S-3-G4-IGFR		CADPGACSSLNFYDWFFVQMRG	1.9	5	--	--	--	--
A6S-3-H8-IGFR		NPTSVQQYGVNFYDWFFVNVL	2.1	4	--	--	--	--
A6S-3-E3-IGFR		RPSLPEVRPGNFYDWFFQSVR	2.0	4	--	--	--	--
A6S-3-D9-IGFR		SLQGADEFQQGNFYDWFFVSEL	1.9	4	--	--	--	--
A6S-2-A1-IGFR		LSSRGRTVMRNFYDWFFVAQVV	1.7	4	--	--	--	--
A6S-1-H4-IGFR		HKSWTMSPLNFYDWFFVAQVE	3.1	3	--	--	--	--
A6S-3-C1-IGFR		RPVIGGGCTRNFYDWFFVAQMI	1.8	3	--	--	--	--
A6S-3-B10-IGFR		YDQDPYYWGLNFYDWFFVREVA	1.7	3	--	--	--	--
			16	3	--	--	--	--

FIG. 1F-3

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Clone	Parental/Design	Comparisons			
		E-Tag	IGFsR	IR	IR/IGFR
	Sequence	19.0	4.0	--	--
	<u>YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV</u>	22.6	19.8	26.5	0.7
A6L-3-D1-IR	<u>ORGMLVRGRISHGAGKIAYEPPDCILGOKACAVNFYDWFV</u>	37.5	3.5	4.2	1.3
A6L-4-H7-IR	<u>ORGMLLGRISDDAGKVASEPSARRGOKVFAFNFYDWFV</u>	38.5	21.1	25.8	1.2
A6L-4-H4-IR	<u>YRGMLVLGRISEGAGKVASEPARAIGQKVFAFNFYDWFV</u>	38.1	5.4	6.0	1.2
A6L-4-E4-IR	<u>ORGMLALGRISDGAGKVASEPPAGIGQKVFAFNFYDWFV</u>	38.6	16.2	18.5	0.9
A6L-4-G7-IR	<u>FRGRLVLGHFSDGAGKVSEPARIGQKVFDVNFYDWFV</u>	34.7	21.8	23.1	0.9
A6L-3-C3-IR	<u>YRGMLVLGRISDGAGKVASEPPARIGQEVADNFYDWFV</u>	33.1	27.8	30.3	1.1
A6L-3-B6-IR	<u>YRGMLVLGRISDGAGEVASEPPARIGQEVFALNFYDWFV</u>	27.6	2.0	2.0	1.0
A6L-4-G11-IR	<u>VPWYAGSGSSSDGAGKVASEPPARIDQKVFAVNFYDWFV</u>	32.0	2.3	2.3	1.0
A6L-4-G12-IR	<u>YRGQLVLGRISYGAQKVGCDDPARIGQKDPPARIGQEVDFV</u>	21.1	2.4	2.4	1.0
A6L-3-A10-IR	<u>QRGLVLGRFSDGAGNVASEPPAGIGQEVFPVNFYDWFV</u>	3.1	2.4	2.4	1.0
A6L-4-E12-IR	<u>QRGMVLVLGRISDGAGKVAEAPPDCILGOKVCAVNFYDWFV</u>	30.1	3.8	3.8	1.0
A6L-4-E10-IR	<u>QRGMRLVLGRISDGAGKVASELPPRIGQKDFAVNFYDWFV</u>	37.9	4.7	4.7	1.0
A6L-4-G8-IR	<u>QRGMVLVLGSISDGAGKVAYEAPARIGQTVFAVNFYDWFV</u>	29.5	5.7	5.7	1.0
A6L-3-C12-IR	<u>OPWCAGSGRIYDAGKVASEPPAHIGQEVFAVNFYDWFV</u>	35.4	9.6	9.6	1.0
A6L-4-H11-IR	<u>QRGMVLVLDRISDGAGKVASGPAPARIGQNVLAVNFYDWFV</u>	31.6	10.5	10.5	1.0
A6L-4-F10-IR	<u>YRGMLVVGRISDGAGKVASQPPARIGQKVFAVNFYDWFV</u>	39.8	12.9	12.9	1.0
A6L-4-E9-IR	<u>YRGMLVLGRISDGAGKVASVPPAHIGQKVFAFNFYDWFV</u>	38.2	14.6	14.6	1.0
A6L-4-H8-IR	<u>QHGMVLVLGRISVGAGKVPSEPARIGHKVFDVNFYDWFV</u>	29.0	17.5	17.5	1.0
A6L-3-A11-IR	<u>YSGWAGSGSFSDGAGKVASEPPARISQEVLADNFYDWFV</u>	35.7	18.4	18.4	1.0
A6L-4-F9-IR	<u>YRGMLVLGRISDGAGKVASEPPARIARKVFADNFYDWFV</u>	29.5	21.4	20.7	1.0
A6L-4-G2-IR	<u>YHGLDLGRISVGAGKVASDPARIGQKVFAFNFYDWFV</u>	28.7	21.6	21.6	1.0
A6L-4-E8-IR	<u>YRGQAGSGGVGSLTVAGKVASDPARIGQKVFAFNFYDWFV</u>	30.0	22.1	22.1	1.0
A6L-4-H10-IR	<u>HRGMLVLGRISEGAGNVDEPPARIGQNVFAGNFYDWFV</u>	37.1	22.6	22.6	1.0
A6L-4-G9-IR	<u>QRGMPVLGRISDGAGKVSEPPARIARKVFADNFYDWFV</u>	28.6	23.6	24.4	1.0
A6L-4-F7-IR	<u>QGGLLVTGRISDGAGKVASEPPGGIGQKVFAGNFYDWFV</u>	38.4	26.5	26.5	1.0
A6L-4-E11-IR	<u>YPWYGGSGSTYLDGAGKVASEPPARIDQQVFAGNFYDWFV</u>				

FIG. 1G-1

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Clone Parental/Design	Sequence	Comparisons			
		E-Tag	IGF ₃ R	IR	IGFR/IR IR/IGFR
A6L-4-H9-IR	YRGMLVLGRISDAGKVASEPPARIGQKVFAVNFYDWFV	19.0	.4.0	--	--
A6L-4-E1-IR	YRAMLVLRISDVAGIVDSEPPTRIGQKVFAGNFYDWFV	37.5	27.3	27.3	1.0
A6L-3-A5-IR	YRGMLVLGRIS <u>Q</u> GAGNVASEPSSRIGQKVFAGNFYDWFI	35.4	32.6	31.4	1.0
A6L-4-G4-IR	YRGMLVLGRISDGAGKVVDYEPPARIGQKVFAGNFYDWFV	38.3	34.6	35.5	1.0
A6L-4-H2-IR	YRGIIIFQGRIPDGAGKVASEPPTRIGERVEAVNFYDWFV	30.4	17.7	15.2	1.2
A6L-4-E6-IR	QGGMPVLGRISDGAGKVASEPPARIGQKVFAGNFYDWFV	36.1	4.2	3.6	0.9
A6L-4-H5-IR	YRGMLVLGRIQDGAGKVASEPPARIGQKVFTGQVFAVNFYDWFV	28.6	24.1	22.7	1.1
A6L-4-H3-IR	QRGMLVLGGVSDGAGKVASDPASSIGQKVFVNFYDWFV	37.2	24.6	23.1	1.1
A6L-4-E5-IR	YPGMILIDRISDGASKWVSEPPASIGQKVFVNFYDWFV	42.1	30.6	24.4	1.3
A6L-3-C5-IR	YRGMLVLDRISDGAGKVASE <u>Q</u> PARIGQEVYAVNFYDWFV	42.2	21.9	17.5	1.2
A6L-4-G6-IR	YRGMLDLGRISGGVGKVASESPARIGQKVYAVNFYDWFV	29.8	4.3	2.8	0.7
A6L-3-D4-IR	ORGMMVLGRISDGAGEVAESEKVFAVNFYDWFV	39.9	12.4	8.4	1.5
A6L-3-A7-IR	ORGMLVLGRVSDGAGKVDSAPPARIGQKVFAGNFYDWFV	31.0	21.2	14.0	1.5
A6L-3-A6-IR	ORGMLVLGRMSDGAGKVAFEPARIGQRGAGNFYDWFV	25.5	12.3	8.8	1.4
A6L-4-E7-IR	QRGTIVLGRISDGAGKAASEPPARIGQNVFVNFYDWFV	38.4	12.5	7.1	1.7
A6L-3-C6-IR	ORGMLVLDRISDGAGKVAAEPARIGQKVFALNFYDWFI	28.8	10.9	6.7	1.6
A6L-4-F5-IR	ORGMLVLGRISDGAGEWASEPPARIGEKVYAVNFYDWFV	33.8	6.3	4.1	1.5
A6L-3-B7-IR	ORGILVRGRISDGAGKVGEPPARSGEKVFAVNFYDWFI	27.6	9.4	5.0	1.9
A6L-4-F4-IR	QLGMVVLGRISDGSGKAASEPARIOSQKVFAVNFYDWFV	38.9	17.6	9.4	1.9
A6L-4-E3-IR	ORGMLVLGRISDGDKVASEPPARIGQRFVNFYDWFV	38.0	6.9	3.8	1.8
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFVNFYDWFV	31.0	31.0	1.8	17.0
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKVFVNFYDWFV	26.0	16.0	1.3	13.0
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASEPARIQKVSAVNFYDWFV	27.0	26.0	2.0	13.0

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FIG. 1G-2

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Clone Parental/Design	Sequence	Comparisons		
		E-Tag	IGF&R	IR
A6L-4-F8-IGFR	<u>YRGMLVLGRISDGAGKVASEPPARIGQKVFAYNFYDWFV</u>	19	4	--
A6L-2-G9-IGFR	YRGMMVQGRISDGAGKVASVS ^P VRIGQKVIAVNFYDW ^F V	26	28	--
A6L-4-E7-IGFR	YRGRLGLGRISDVAGKVACDPSARIQKVLPVNFYDW ^F V	39	22	--
A6L-4-G10-IGFR	YRGMLVLGRISDGAGRVASEPQARIQKVFAVNFYDW ^F V	23	22	--
A6L-2-E9-IGFR	OGGMLVPGRISDGAGKVASQP ^P PARIGPKGFAGNFYDW ^F V	19	22	--
A6L-2-D6-IGFR	YRGMRVLGRISDGAGKVASEPPTHIQQKVFPVNFYDW ^F V	38	21	--
A6L-3-H12-IGFR	YRGMLVLGRISDGAGKVSEPAARIQKVFAVNFYDW ^F V	34	21	--
A6L-4-A7-IGFR	YRGQQGMVLGRISDGAGKVASEPPGRIGQKVFPVNFYDW ^F V	24	21	--
A6L-4-B8-IGFR	YRGMLGLGRITGGAGKVASEPPDRIGQHVFVDNFYDW ^F V	20	20	--
A6L-4-G7-IGFR	DGMILVLGRISDGAGNVASEAPARIQKVFAVNFYDW ^F V	20	19	--
A6L-2-D9-IGFR	YRGMRVRGRISDGAGKAASDPRARIGQTVLDVNFYDW ^F V	19	19	--
A6L-4-F7-IGFR	YRGMWVLGRISYGAGKVAYEPPARMGQKGFAVNFYDW ^F V	38	18	--
A6L-4-E12-IGFR	YRGMLVGGRIGAGGKVASEPPARIGQKVFAVNFYDW ^F V	18	18	--
A6L-4-H7-IGFR	YRGMLGLGGISDGAGKVASEP ^P PARNGQKVFAVNFYDW ^F V	15	13	--
A6L-4-H12-IGFR	YRGMLALGRISSEGAGKVASEPPARIGQNVFAVNFYDW ^F V	14	13	--
A6L-2-R4-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVLAVNFYDW ^F V	13	12	--
A6L-3-D10-IGFR	YPGMLVPGRISDGAGEGATDPPPRIGQKVFAFNFYDW ^F V	17	4	--
A6L-2-F6-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKIFAVNFYDW ^F V	16	4	--
A6L-2-B11-IGFR	YRGVLVLGRVSDDGVGKVASEPPAHRGQRVFGVNFYDW ^F V	26	3	--
A6L-1-B7-IGFR	YRRMLVLGRISDGAGANVASGPPDRIGQKVFAGNFYDW ^F V	23	3	--
A6L-1-D8-IGFR	YRRMLALGRFSDTGDVASEPPAHIGOKVVAVNFYDW ^F V	23	3	--
A6L-0-A11-IGFR	YRGMLVVRGRIFDGPGRKVASEPPARIGQKVFAVNFYDW ^F V	15	4	--
A6L-3-B7-IGFR	YRGMLLILGRISDGAGKVASEPPARVGQDQVAVNFYDW ^F V	9	3	--
A6L-1-G7-IGFR	YPGRLVGGRISDGVGKVASEPPGRIGOKVFAVNFYDW ^F V	20	2	--
A6L-1-B9-IGFR	QRGLLLVGRIFDGAQKVASDPPARIGQKDFAADNFYDW ^F V	18	2	--
A6L-1-C9-IGFR	YRGMLVLGRISDGAGKVAFEPARIGQNVFAVNFYDW ^F V	18	2	--
A6L-0-G10-IGFR	YRCMPVLGRISDGAG#VASDRPARIGQKVFAVNFYDW ^F V	18	2	--
A6L-1-G8-IGFR	YRGRLVLGRISDGAGKVAAEPASMDSKVFAGNFYDW ^F V	15	2	--

FIG. 1H

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Clone Design	Sequence	Comparisons			
		IGFRsR	IR	IGFR/IR	IR/IGFR
	Ratios over Background	E-Tag	--	--	--
E4Da-1-B8-IR	<u>GFREGQFYDWFVAQVT</u>	40.7	1.0	12.3	0.1
E4Da-3-E5-IR	GFREGQFYDWFLAQVT	39.6	2.0	1.5	1.3
E4Da-1-A1-IR	GFREGDFYEWFVAQVT	48.7	44.9	31.4	1.4
E4Da-2-D9-IR	GFREGQFYEWFAAQVT	22.9	3.3	2.4	1.4
E4Da-1-B3-IR	GFREGTFYDWFWVAQVT	41.8	38.6	26.5	1.5
E4Da-1-A6-IR	GFREGNFYDWFEAQVT	56.3	51.2	32.6	1.6
E4Da-1-A10-IR	GFREGAFYDWFEAQVT	48.9	42.2	26.5	1.6
E4Da-1-A8-IR	GFREGAFYDWFWVAQVT	46.9	41.5	26.2	1.6
E4Da-1-B1-IR	GFREGKFYQWFEAQVT	44.1	31.1	19.7	1.6
E4Da-2-C9-IR	GFREGCDFYDWFWQAQVT	34.0	8.1	4.8	1.7
E4Da-1-A3-IR	GFREGTFYEWFWVAQVT	45.3	40.3	22.5	1.8
E4Da-1-A9-IR	GFREGNFYDWFWVAQVT	46.9	41.0	22.5	1.8
E4Da-3-F3-IR	GFREGQFYDWFLAQVT	37.2	14.1	8.0	1.8
E4Da-2-D3-IR	GFREGQFYDWFWQAQVT	35.1	16.3	8.7	1.9
E4Da-2-D6-IR	GFREGEFYDWFWQAQVT	33.2	5.6	2.8	2.0
E4Da-3-F10-IR	GFREGQFYDWFWQAQVT	27.8	4.5	2.3	2.0
E4Da-2-D5-IR	GFREGGYFYEWFWQAQVT	43.8	23.8	11.4	2.1
E4Da-3-F4-IR	GFREGDFYQWFEAQVT	25.9	7.6	3.7	2.1
E4Da-3-E3-IR	GFREGGSFYGWFQQAQVT	34.6	4.0	1.9	2.1
E4Da-3-F8-IR	GFREGGSFYAWFWQAQVT	20.9	16.0	7.4	2.2
E4Da-2-C1-IR	GFREGQFYDWFWVAQVT	43.1	11.6	5.0	2.3
E4Da-1-B4-IR	GFREGGIFYEWFWVAQVT	45.3	6.6	2.9	2.3

FIG. 11-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
E4D α -4-H5-IR	<u>GFREGGSFYEWFWFVAQVT</u>	47.2	36.0	14.7	2.4	0.4	
E4D α -1-B12-IR	GFREGSFYEWFWFQAQVT	47.6	33.4	13.8	2.4	0.4	
E4D α -4-G2-IR	GFREGNFYDWFWFAAQVT	23.4	20.4	8.6	2.4	0.4	
E4D α -3-F9-IR	GFREGDFYDWFWFVAQVT	36.2	15.6	6.3	2.5	0.4	
E4D α -4-G6-IR	GFREGDFYQWFVAQVT	26.0	4.9	2.0	2.5	0.4	
E4D α -4-H9-IR	GFREGGFFYDWFWFVAQVT	47.8	24.8	9.5	2.6	0.4	
E4D α -2-C10-IR	GFREGCDFYGWFWQAQVT	42.4	23.2	9.0	2.6	0.4	
E4D α -1-B2-IR	GFREGGVFYDWFWFVAQVT	39.4	18.7	7.2	2.6	0.4	
E4D α -3-F12-IR	GFREGGGFYEWFWQAQVT	38.9	16.6	5.6	3.0	0.3	
E4D α -2-D11-IR	GFREGGSFYDWFWQAQVT	40.2	11.1	3.3	3.4	0.3	
E4D α -4-H2-IR	GFREGNFYEWFWQAQVT	37.8	33.9	8.2	4.1	0.2	
E4D β -4-A12-IR	GFREGKFYDWFWFQAQVT	41.1	8.3	28.7	0.3	3.5	14/122
E4D β -4-A10-IR	GFREGEFYEWFWFVAQVT	5.8	1.2	2.4	0.5	2.0	
E4D β -4-E10-IR	GFREGGRFYDWFWFVAQVT	9.6	1.2	2.2	0.5	1.8	
E4D β -4-B11-IR	GFREGTFYDWFWFVAQVT	36.1	15.2	26.9	0.6	1.8	
E4D β -4-C10-IR	GFREGGEFYEWFWQAQVT	27.8	13.3	23.7	0.6	1.8	
E4D β -4-E8-IR	GFREGDFYEWFWFVAQVT	28.7	16.7	28.2	0.6	1.7	
E4D β -4-G7-IR	GFREGHFYDWFWQAQVT	30.9	14.7	24.7	0.6	1.7	
E4D β -4-C8-IR	GFREGGEFYDWFWFVAQVT	35.5	22.5	32.9	0.7	1.5	
E4D β -4-A8-IR	GFREGGSFYDWFWFVAQVT	31.2	14.5	22.2	0.7	1.5	
E4D β -4-A9-IR	GFREGGSFYDWFWGAQVT	35.8	9.0	13.1	0.7	1.5	
E4D β -4-G11-IR	GFREGTFYDWFWFQAQVT	28.9	9.7	13.6	0.7	1.4	
E4D β -4-B9-IR	GFREGNFYEWFWFVAQVT	27.2	9.1	12.5	0.7	1.4	
E4D β -4-F10-IR	GFREGGSFYNWFWQAQVT	41.1	27.2	36.1	0.8	1.3	
E4D β -4-D12-IR	GFREGCDFYDWFWFVAQVT	35.9	27.0	35.2	0.8	1.3	
E4D β -4-B8-IR	GFREGGAFYDWFWFAAQVT	38.5	25.5	33.7	0.8	1.3	
E4D β -4-G10-IR	GFREGGSFYDWFWFVAQVT	34.1	19.3	25.7	0.8	1.3	
E4D β -4-D9-IR							

FIG. 11-2

Clone Design	Sequence	Comparisons					
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
E4D β -4-F8-IR	<u>GFREGNFYDWFWFVAAQVT</u>	--	39.3	35.6	44.4	0.8	1.2
E4D β -4-E12-IR	<u>GFREGGSFYDWFWAAQVT</u>	--	40.2	27.8	33.4	0.8	1.2
E4D β -4-H12-IR	<u>GFREGGAFYDWFWEAQVT</u>	--	41.2	27.1	32.3	0.8	1.2
E4D β -4-C9-IR	<u>GFREGQFYDWFWAAQVT</u>	--	38.0	22.5	27.6	0.8	1.2
E4D \square -4-H9-IR	<u>GFREGGNFYDWFWAAQVT</u>	--	38.7	33.3	36.6	0.9	1.1
E4D \square -4-G9-IR	<u>GFREGCDFYDWFWAAQVT</u>	--	10.9	4.9	5.6	0.9	1.1
E4D β -4-F12-IR	<u>GFREGGSFYEWFWEAQVT</u>	--	14.8	5.9	6.1	1.0	1.0
E4D β -4-F9-IR	<u>GFREGGGFYDWFWLAQVT</u>	--	39.3	31.3	28.3	1.1	0.9
E4D β -4-F7-IR	<u>GFREGGGFYAWFWAAQVT</u>	--	31.0	22.2	19.5	1.1	0.9
E4D β -4-B7-IR	<u>GFREGGGFYEWFWAQVT</u>	--	--	--	--	--	--

FIG. 11-3

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
E4D-2-E7-IGFR	<u>GFREGNFTYDWFVFAQVT</u>	--	20.8	22.8	--	--	
E4D-2-C11-IGFR	GFREGDFYDWFRFAQVT	21.5	22.6	--	--	--	
E4D-2-B1-IGFR	GFREGGSFYDWFWVAQVT	22.0	22.5	--	--	--	
E4D-2-D10-IGFR	GFREGGFYDWFWFAQVT	20.6	22.1	--	--	--	
E4D-2-A9-IGFR	GFREGGDFYDWFWVAQVT	17.4	21.5	--	--	--	
E4D-2-E5-IGFR	GFREGGDFYDWFWFAQVT	24.2	21.2	--	--	--	
E4D-2-H9-IGFR	GFREGGFYDWFWVAQVT	19.1	20.7	--	--	--	
E4D-1B-C4-IGFR	GFREGGDFYDWFWFAQVT	24.3	20.5	--	--	--	
E4D-2-E10-IGFR	GFREGNFTYDWFVFAQVT	21.0	20.5	--	--	--	
E4D-2-F4-IGFR	GFREGGNFTYDWFVLAQVT	25.0	20.2	--	--	--	
E4D-2-C10-IGFR	GFREGGHFYDWFWFAQVT	22.8	20.1	--	--	--	
E4D-3-D8-IGFR	GFREGQQFYEWFWFAQVT	21.1	19.8	--	--	--	
E4D-3-F9-IGFR	GFREGGSFYEWFWFAQVT	22.6	19.7	--	--	--	
E4D-1B-E5-IGFR	GFREGGHFYDWFWFAQVT	24.2	18.8	--	--	--	
E4D-2-F3-IGFR	GFREGQQFYEWFWFAQVT	23.6	18.0	--	--	--	
E4D-3-D5-IGFR	GFREGQFYEWFWFAQVT	22.2	18.0	--	--	--	
E4D-3-G10-IGFR	GFREGQFYDWFWFAQVT	22.1	17.6	--	--	--	
E4D-2-F6-IGFR	GFREGQFYDWFWVAQVT	24.6	17.5	--	--	--	
E4D-2-F7-IGFR	GFREGQFYQWFVFAQVT	19.0	17.5	--	--	--	
E4D-3-B7-IGFR	GFREGNFTYDWFVFAQVT	23.0	16.4	--	--	--	
E4D-1B-C12-IGFR	GFRDGSFYDWFWVAQVT	23.0	16.1	--	--	--	
E4D-3-B1-IGFR	GFREGHFYEWFWFAQVT	21.6	16.0	--	--	--	
E4D-2-E2-IGFR	GFREGDFYDWFSQAQVT	21.9	14.1	--	--	--	
E4D-2-D1-IGFR	GFREGHFYDWFDAQVT	24.5	13.2	--	--	--	
E4D-1-D4-IGFR	GFREGGYFYDWFWFAQVT	18.9	12.4	--	--	--	
E4D-1B-A10-IGFR	GFREGDFYDWFEAQVT	23.9	10.8	--	--	--	
E4D-1B-A3-IGFR	GFREGGTFYDWFWVAQVT	22.2	10.8	--	--	--	
E4D-1-B5-IGFR	GFREGHFYDWFWFAQVT	19.0	10.8	--	--	--	

FIG. 1J-1

Clone Design	Sequence	Ratios over Background				Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
E4D-1B-B8-IGFR	GFREGNFYDWFVVAQVT	--	23.8	10.7	--	--	
E4D-1-G7-IGFR	GFREGDFYAWFMAQVT	--	14.3	10.5	--	--	
E4D-1B-A11-IGFR	GFREGGNFYEWFLAQVT	--	24.0	10.0	--	--	
E4D-1-C3-IGFR	GFREGGSFYDWFDAQVT	--	15.8	9.3	--	--	
E4D-2-H1-IGFR	GFREGGNFYDQFVAQVT	--	19.6	4.9	--	--	
E4D-1-C2-IGFR	GFREGHFYEWFAAQVT	--	11.5	4.5	--	--	
E4D-1B-A12-IGFR	GFREGGNFYDWFVVAQVT	--	18.4	3.5	--	--	
E4D-1B-A1-IGFR	GFREGKFYDWFVVAQVT	--	22.5	2.9	--	--	
E4D-2-A3-IGFR	GFREGGMFDVQLLAQVT	--	22.7	2.1	--	--	
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FIG. 1J-2

Clone Design	Sequence	Comparisons			
		E-Tag	Background	IGFR/R	IR/IGFR
Parental	<u>XXXXXXXXXXHENFYDWFVRQVSXXXXXX</u> VTFSAVTHENFYDWFVRQVS	--	--	17.5	16.3
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVS <u>QYFGRV</u>	29.8	--	--	1.1
H2CA-4-E10-IR	ORLSLHE <u>QFYDWFVGQVSPLGAGG</u>	37.7	2.2	18.1	0.1
H2CA-4-G3-IR	GGGKVNFHEDFYGMFW <u>QQFSGVGSDR</u>	31.2	4.4	18.8	0.2
H2CA-3-A11-IR	LVGDA <u>PFHEDFYDWFAQRQVFGCCQE</u> Q	36.1	13.4	25.7	0.5
H2CA-4-F8-IR	TGAEV <u>SFHENFYDWFDRQYSSWLDRD</u>	35.6	12.1	22.0	0.5
H2CA-4-G4-IR	<u>QPHSSRLHESFYDWFDRQWPWYALDR</u>	36.0	21.1	33.5	0.6
H2CA-4-F4-IR	SRALAAVHE <u>QFYDWFVRQVSGLDWGY</u>	37.1	23.3	34.3	0.7
H2CA-4-H10-IR	QPKDGT <u>LHENFYDWFVRQVSSSGWVG</u>	39.8	25.0	35.6	0.7
H2CA-4-F1-IR	RGRLL <u>IQHLHEDFYDWFFLRQVSQMGCGGS</u>	33.5	5.1	6.6	0.8
H2CA-3-D5-IR	<u>ORGAPKSDENFYDWFVRQVLRFGEND</u>	36.1	19.6	25.1	0.8
H2CA-4-E11-IR	AARTSL <u>FHEDFYEWFDRQVRQEGMWG</u>	39.3	24.3	31.9	0.8
H2CA-3-B6-IR	GTSNH <u>SLHENFYDWFVRQLISSVQSSG</u>	35.9	9.9	12.1	0.8
H2CA-3-A9-IR	VSHVHL <u>FHENFYDWFVRQLAAEGFSG</u>	37.3	30.1	36.2	0.8
H2CA-4-H5-IR	GROD <u>SGLHEHFYDWFWSRQVQGEVALIG</u>	38.6	35.4	37.3	1.0
H2CA-3-C9-IR	<u>SNDERQFHETFYDWFVRQVSADGADR</u>	29.3	5.1	5.6	0.9
H2CA-3-A10-IR	LSTE <u>QRFHEKFYDWFVHQVSTSGGGT</u>	37.2	16.9	19.1	0.9
H2CA-3-A3-IR	SLSRE <u>QFHENFYDWFARQVSELEGTV</u>	29.2	28.6	32.2	0.9
H2CA-4-G8-IR	IPGRR <u>SSLHENFYDWFVRQVSPGGGS</u> A	32.4	29.1	31.6	0.9
H2CA-4-G9-IR	TOKAQ <u>OSLDEKFYDWFVRQVSGGGLTG</u>	36.1	34.4	36.4	0.9
H2CA-4-G10-IR	VSOL <u>QSDFFHENFYGMWARQIAQQAEWT</u>	34.2	35.5	37.7	0.9
H2CA-4-H7-IR	NGTS <u>QALHQNFYDWFQAQQISGSEPGP</u>	37.0	36.0	40.0	0.9
H2CA-4-F9-IR	VGQS <u>VTFHGDFYDWFDRQILGSQEEFG</u>	37.5	36.7	39.5	0.9
H2CA-4-F7-IR	TIDHH <u>HPPLHEQFYDWFARQVSDELSLG</u>	37.7	37.6	39.9	0.9
H2CA-3-D10-IR	PNVGYAF <u>HENFYDWFVRQVSEW1QFG</u>	18.7	3.6	3.5	1.0
H2CA-3-B1-IR	SRGSGV <u>FPHESFYNWFDRQVSGSAGGG</u>	26.5	21.4	21.5	1.0
H2CA-3-A5-IR	QPVS <u>GSVHERFYDWFVRQVSGSAGGG</u>	32.9	22.9	22.4	1.0
H2CA-4-F10-IR	ASQ <u>LPVVYENFYEWFDRQVSLDAQRE</u>	26.6	27.7	28.5	1.0

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FIG. 1K-1

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Clone Design	Sequence	Comparisons			
		E-Tag	IGF&R	IR	IGFR/IR
H2CA-3-D9-IR	<u>XXXXXXXXXXHENFYDWFVRQVSXXXXXX</u>	36.6	30.6	30.9	1.0
H2CA-3-C2-IR	VSGRGA <u>FHENFYDWFVRQFRDEQDT</u>	38.3	30.7	31.0	1.0
H2CA-4-G1-IR	ARPPTV <u>HENFYDWFVRQVSETWRQD</u>	34.1	30.7	30.4	1.0
H2CA-4-E2-IR	QGGDR <u>LHERFYDWFDRLVSSDSTGE</u>	33.9	31.0	31.8	1.0
H2CA-4-H9-IR	QHIAAGL <u>HENFYDWFIRQVSGVNVP</u> A	38.9	31.1	31.4	1.0
H2CA-3-D2-IR	QPNDG <u>LLHENFYDWFVRQVSNAVDGG</u>	33.0	31.1	29.8	1.0
H2CA-3-B3-IR	PVEFTVYHDNF <u>YDWFARQVSDFGLGQF</u>	35.3	31.4	30.0	1.0
H2CA-4-G11-IR	FCVQ <u>ASIHENFYDWFVRQVAENQVFS</u>	37.9	31.9	31.0	1.0
H2CA-4-F2-IR	GRPRGS <u>FHENFYDWFARQVSGDGAGT</u>	38.1	32.0	31.9	1.0
H2CA-3-C5-IR	IVGASL <u>CHESFYDWFACQVTNLQSQG</u>	36.9	32.3	31.6	1.0
H2CA-3-B2-IR	IGLR <u>QMFHENFYDWFAREVSKEAGDG</u>	36.6	32.7	32.5	1.0
H2CA-3-B11-IR	LGGA <u>TEGHGNFYDWFVRQVSLDVGG</u> E	35.5	32.8	33.3	1.0
H2CA-4-G2-IR	LNAL <u>QQLHENFYDWFGRQVSATPPGG</u>	35.9	33.0	33.4	1.0
H2CA-3-A4-IR	VGNCD <u>TFPENFYDWFACQVSELGGMN</u>	33.3	33.0	32.9	1.0
H2CA-4-H3-IR	FSQDG <u>NFHENFYDWFDRQLSLVAGGT</u>	39.5	33.7	33.7	1.0
H2CA-4-G5-IR	PAGR <u>NALHESFYDWFVRQVSEFQLGA</u>	35.3	34.0	35.6	1.0
H2CA-4-E8-IR	DRLRA <u>RFENFYDWFDRQVSQGSMP</u>	35.7	34.7	34.9	1.0
H2CA-4-G6-IR	VLGVA <u>QFDKFYDWFARQVSQLESAG</u>	36.2	35.0	33.5	1.0
H2CA-3-B7-IR	GVVG <u>GAFHEQQFYDWFDRQVSAAFKGD</u>	37.6	36.5	35.3	1.0
H2CA-3-B4-IR	DESEMRL <u>HEQQFYDWFARLVSLEGGSA</u>	39.8	36.5	35.1	1.0
H2CA-3-C7-IR	EGGGVA <u>IHENFYDWFDRQVSLQGWSD</u>	40.2	36.7	35.9	1.0
H2CA-4-E5-IR	SRIVS <u>RFHENFYDWFVRQVSGDAPVQ</u>	37.3	37.0	36.3	1.0
H2CA-4-E7-IR	IPAGAQ <u>LHENFYDWFARQVSGEDGGA</u>	39.7	37.6	37.6	1.0
H2CA-3-B9-IR	GSSAAG <u>GFDEQFYDWFDRQVSEAFRDG</u>	39.4	37.7	37.6	1.0
H2CA-4-F5-IR	RLALRT <u>FHQDFYDWFVRQVAAEDTD</u> P	38.8	38.0	37.8	1.0
H2CA-3-B10-IR	QGSFAV <u>LHENFYDWFARQVSGVEGLA</u>	41.9	38.9	38.0	1.0
H2CA-3-A12-IR	QGNM <u>MSALHENFYDWFVRQVSEADRV</u> D	37.8	7.3	6.3	0.9
H2CA-3-A8-IR	VAYPALL <u>HEQQFYDWFVRQVSAVAGTT</u>	36.8	22.5	19.2	0.9
	PDTINS <u>QHKNHFYDWFVRQSVGTSS</u>				

FIG. 1K-2

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Clone Design	Sequence	Comparisons			
		E-Tag	Ratios over Background	IGF&R	IR
H2CA-3-D12-IR	<u>XXXXXXXXXX</u> <u>XOXOXOXFHENFYDWFVROVSXXXXXX</u>	--	36.8	34.1	29.6
H2CA-3-B5-IR	SEDVDSRHENFYDWFVROVS <u>GIGLQD</u>	38.8	35.2	30.5	1.2
H2CA-4-E1-IR	PAPADA <u>FDHNFYDWFARQLSATTTIQ</u>	29.8	12.5	11.3	1.1
H2CA-3-D3-IR	MVQRISIHENFYDWFVROQISGSAVPP	33.1	29.9	27.5	1.1
H2CA-4-E3-IR	GNVRGGQFHGQFYDWFARQVGSEGDA	33.3	32.3	30.2	1.1
H2CA-4-E12-IR	PDAEK <u>QFHETFYGWFVROQISEDANS</u>	36.0	32.4	29.4	1.1
H2CA-3-A6-IR	FGRGVHCDENFYDWFVFC <u>QVSGALLEG</u>	34.0	33.1	30.6	1.1
H2CA-4-E9-IR	ETPLTELHE <u>QFYDWFVROQVSSAVPSD</u>	38.8	33.7	29.7	1.1
H2CA-4-F3-IR	QHRGPHFFHEDFYDWFVROQVSSAVPSD	41.0	34.2	32.0	1.1
H2CA-4-H6-IR	<u>RDGPGLFHNDNFYDWFDFRLVSAWDQE</u>	37.1	34.5	30.8	1.1
H2CA-4-H2-IR	QAAGVGCNKDFYAWFACQVREDFAKA	41.8	35.3	32.8	1.1
H2CA-3-D4-IR	<u>RNWNLQFNENFYDWFDRQVSALRGGG</u>	38.7	35.5	32.3	1.1
H2CA-3-D1-IR	GAGGGRDFDEFYDWFVROQVSGQVTSG	34.5	35.5	31.3	1.1
H2CA-3-C1-IR	SPEGNLVHD <u>DQFYDWFVROLSSTSAGT</u>	39.9	36.1	32.9	1.1
H2CA-3-D8-IR	<u>QGGLGDFDEFYDWFARQVSRRDRA</u> D	37.8	36.7	33.1	1.1
H2CA-4-H4-IR	LSQQGVGFQENFYEWFERQVSGWDGRD	38.5	37.0	33.7	1.1
H2CA-4-F6-IR	VEFERSRCHDNFYDWFFFCQVSGQADGG	38.7	37.5	35.2	1.1
H2CA-4-E4-IR	LLASRAFHENFYDWFARQVS <u>GPG</u>	38.6	38.0	34.7	1.1
H2CA-3-C11-IR	VPDAAQIFHESFYDWFVROQASAGGRAD	40.3	38.3	36.1	1.1
H2CA-3-C4-IR	ANQMGRFHDNFYDWFDRQVSRYERGT	41.9	38.4	35.0	1.1
H2CA-4-E6-IR	PSRKDGLHQSFYDWFARQV <u>QDMEGRA</u>	39.3	38.8	35.8	1.1
H2CA-3-D7-IR	QAVTRRFHENFYDWFARQVSEEGGWS	42.5	39.2	35.5	1.1
H2CA-3-A7-IR	GYAVGQOQANFYDWFVROQV <u>DGMNSGG</u>	35.3	15.2	11.6	1.3
H2CA-4-G12-IR	GHORDLLHESFYDWFVROQVSEAE <u>EGGG</u>	37.6	19.4	15.1	1.3
H2CA-3-D6-IR	DRPSSFIHENFYEW <u>ARQVSQSGSSG</u>	39.4	36.2	27.6	1.3
H2CA-4-H12-IR	ERTAETI <u>LHEQFYDWFVROQVSAMDGES</u>	40.0	38.4	29.3	1.3
H2CA-3-D11-IR	LTS <u>QLLSHEDFYDWFVROQSVGGSG</u>	38.1	32.9	27.2	1.2
H2CA-3-C12-IR	PDRSDR <u>DDNFYDWFVROQSVINED</u>	38.5	38.4	31.7	1.2

FIG. 1K-3

Clone Design	Sequence	Comparisons				21/122
		IGF1R	IR	IGFR/IR	IR/IGFR	
H2CA-4-G7-IR	<u>RAGGVGLHDNFYDWFVRQVSGGDSGP</u>	35.9	34.7	23.7	1.5	0.7
H2CA-3-C6-IR	<u>ADCYVQLHENFYDWFRROVCNLQEGM</u>	38.7	37.6	28.2	1.3	0.7
H2CA-3-B8-IR	<u>RQGHAGFHDNFYDWFVRQVSGSTPQV</u>	37.8	19.6	9.9	2.0	0.5
E-Tag	--	--	--	--	--	
IGFsR	--	--	--	--	--	
Background	--	--	--	--	--	

FIG. 1K-4

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Clone Design	Sequence	Comparisons					
		E-Tag	Background	IGFeR	IR	IGFR/IR	IGFR
Parental	<u>XXXXXXXXXXFYDWFVHQVSXXXXXX</u>	29.8	17.5	16.3	1.1	0.9	--
H2CA-4-H6-IGFR	VTFITSAYFHENFYDWFAGQVSDPWICW	8.6	9.5	0.6	16.0	0.1	--
H2CA-4-F-IGFRS	GIISQSCPESFYDWFGRQLSLQSSEQ	4.9	10.5	0.7	14.6	0.1	--
H2CA-4-H8-IGFR	VGRASGFPENFYDWFGRQLSLQSSEQ	5.5	9.7	0.8	12.3	0.1	--
H2CA-4-F11-IGFR	VGYQQGQGDENFYDWFIRQVSGRLGVQ	5.6	9.2	1.0	9.4	0.1	--
H2CA-4-F6-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	3.5	6.8	1.0	6.7	0.1	--
H2CA-4-F10-IGFR	SAAQLFFQESFYDWFRLRQVAESSQPN	3.9	7.3	1.1	6.4	0.2	--
H2CA-1-A3-IGFR	AVRATRFDEAFYDWFVTRQISDGQQGNK	4.9	5.7	1.0	5.9	0.2	--
H2CA-3-C8-IGFR	VNQSGSIHENFYDWFERQVSHQRGVR	7.7	3.8	0.8	5.1	0.2	--
H2CA-2-B9-IGFR	APDPSDFQEIIFYDWFVTRQVSMPGGG	15.1	5.6	1.2	4.8	0.2	--
H2CA-4-H4-IGFR	SSCDGAGHESFYEWFWRQVSGCRSV	9.3	7.0	1.7	4.2	0.2	--
H2CA-4-F7-IGFR	RAGSSDFHEDFYEWFWRQVQLSLKGK	3.9	4.1	1.0	4.2	0.2	--
H2CA-3-D6-IGFR	QAVQPGFHEEFYDWFVHQVSTGVGGG	1.5	3.2	0.8	4.1	0.2	--
H2CA-3-D8-IGFR	SSIGGGFHENFYDWFESRQLSQSPPLK	8.3	9.0	2.2	4.0	0.3	--
H2CA-4-G11-IGFR	QSPVGSSSHEDFYDWFERRQVAQSGAHQ	10.9	7.2	1.8	4.0	0.3	--
H2CA-4-F1-IGFR	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.8	9.5	2.5	3.9	0.3	--
H2CA-3-D7-IGFR	TLDGGSFEEQFYDWFVRLQSYRTNPD	5.8	3.5	0.9	3.8	0.3	--
H2CA-1-A7-IGFR	FYVQWQGHENFYDWFVHQVSGDRVGG	13.3	3.0	0.8	3.7	0.3	--
H2CA-2-B4-IGFR	LRRQAPVEENFYDWFVHQVAGRTCP	8.0	2.2	0.6	3.7	0.3	--
H2CA-2-B3-IGFR	RCGRELYHSTFYDWFDRQVAGRTCP	3.5	4.1	1.1	3.6	0.3	--
H2CA-2-B2-IGFR	CLLLCRFQQNFYDWFVCGQGISRLRPL	7.7	3.8	1.0	3.6	0.3	--
H2CA-3-D4-IGFR	PPLASDLVDQFYDWFVRLQSDSAISG	4.1	3.4	1.0	3.5	0.3	--
H2CA-4-F2-IGFR	GAPVDQLHEDFYDWFVHQVSOAATG	17.6	13.8	4.1	3.4	0.3	--
H2CA-3-D11-IGFR	RSASGLSLPEQFYDWFVHQVLSGTDK	9.3	12.8	4.2	3.0	0.3	--
H2CA-4-H9-IGFR	SRVTTFHENFYDWFVRLQSDSAISG	12.2	6.9	2.3	3.0	0.3	--
H2CA-2-B11-IGFR	DERGGKFREDFYDWFVROVSESRFGQ	8.7	5.6	1.9	3.0	0.3	--
H2CA-3-E8-IGFR	RGAVAGFHDDQFYDWFDRQVSRYHKEG	11.9	4.6	1.6	3.0	0.3	--
	AICDAGFHEHFYDWFALQVSDCGRQS	13.2	6.3	2.2	2.9	0.3	--
	LGYQEPFQQNFYDWFVHQVSGAENAG						

FIG. 1L-1

Clone Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGF3R	IR	—	IGFR/IR	IR/IGFR	—
H2CA-3-E6-IGFR	XXXXXXXXXXFHENFYDWFVRQVSXXXXXX	15.7	8.7	3.1	2.8	0.4	0.4	—
H2CA-4-F4-IGFR	WRGHGTFHEDFYDWFVRQVSQQSST	11.5	7.4	3.0	2.5	0.4	0.4	—
H2CA-3-D10-IGFR	GGRGVLHENFYDWFDRQVSLRGADG	9.4	6.8	2.9	2.3	0.4	0.4	—
H2CA-3-E1-IGFR	CNLTAGFHEQFYIWWFAIQVCGDAENA	12.5	6.4	2.8	2.3	0.4	0.4	—
H2CA-2-B6-IGFR	ERGEDDMFHENFYDWFVRQISGRQQGG	18.0	6.2	2.7	2.3	0.4	0.4	—
H2CA-3-E11-IGFR	TNQGVGFYDSFYGWFWVRQIQYGVDSG	4.7	2.2	1.0	2.2	0.5	0.5	—
H2CA-4-H2-IGFR	HLADGQFHEKFYDWFERQISSRCNDL	9.8	9.9	4.8	2.1	0.5	0.5	—
H2CA-3-C11-IGFR	QTFGKSLHENFYDWFVRQVSREEGGD	9.3	3.3	1.6	2.1	0.5	0.5	—
H2CA-2-B8-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	14.6	7.9	3.9	2.0	0.5	0.5	—
	SASTHQFHENFYDWFVRQVSGAQKIL	23/122						

FIG. 1L-2

Clone Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--	--
Parental	XXXXXXXFHXXXXFYXXMXXXXX VFTTSAVFHENFYIDWFVRQVS	29.8	17.5	16.3	1.1	0.9		
H2CB α -3-B12-IR	QSDSGTVHDFYQWFRDT*A	26.0	1.3	20.4	0.1	16.0		
H2CB α -3-D2-IR	WTDVDGFHSQFYRWFWQFQWER	20.6	1.7	12.1	0.1	7.0		
H2CB α -3-D12-IR	VASGHVLHGQFYRWFWDQFAL	24.6	2.1	14.0	0.1	6.7		
H2CB α -3-H5-IR	QARVGNVHQFYEWFREVMQG	16.7	2.4	15.1	0.2	6.3		
H2CB α -3-B6-IR	VGDFCVSHDCFYGWFLRESMQ	31.4	2.5	13.9	0.2	5.6		
H2CB α -3-G11-IR	SGSRPVFHEQFYEWFWFDQLG	22.7	1.4	6.4	0.2	4.7		
H2CB α -3-A6-IR	QFSAGAFHGDFYGWFRALYNG	25.9	1.7	7.1	0.2	4.3		
H2CB α -3-B1-IR	SRFDERLHHQFYEWFRVLINEP	33.4	6.0	25.5	0.2	4.3		
H2CB α -3-F8-IR	DSVNSDLHRAFYGWFAEQWRA	23.0	4.8	19.8	0.2	4.1		
H2CB α -3-E11-IR	GSVDREIHGPFYYSWFSEQLWG	14.0	2.2	8.5	0.3	4.0		
H2CB α -3-G4-IR	SAKTPVLDGIFYMMFEAQSES	24.9	2.2	6.9	0.3	3.2		
H2CB α -3-D3-IR	LUVGRRFHQSFYDWFWVAAGG	23.6	2.6	8.0	0.3	3.1		
H2CB α -3-C1-IR	IMWPCTFQDPFPCWFWQTETQGR	27.0	5.6	16.4	0.3	2.9		
H2CB α -3-C3-IR	VVGPLDIHERFYGMWFHQGGGA	23.3	1.1	3.1	0.4	2.8		
H2CB α -3-G3-IR	VVPKAGFHEAFYEWFRQQRD	23.7	6.7	17.6	0.4	2.6		
H2CB α -3-E4-IR	QSFVTTSVHTRFYAWFASALEM	28.8	8.3	21.9	0.4	2.6		
H2CB α -3-G5-IR	SRGLGLYHSGFYGMWFERQFNQ	26.7	7.0	17.2	0.4	2.5		
H2CB α -3-B11-IR	GADTGAVHRRFLWFEQLSGG	28.0	8.6	19.4	0.4	2.3		
H2CB α -3-A1-IR	PGNRPTFHAFFYRWFREAQGS	31.3	11.3	24.9	0.5	2.2		
H2CB α -3-H1-IR	VAVAWGLHESFYAWFENQFSD	27.2	10.6	23.9	0.4	2.2		
H2CB α -3-F12-IR	GFNTGTFFHDQFYWMFWEAAGG	21.1	6.1	12.7	0.5	2.1		
H2CB α -3-H7-IR	GDGLTAFHQGYFYEWFDIQMVG	21.0	9.7	19.1	0.5	2.0		
H2CB α -3-C12-IR	VGVNROFHTRFYAWFDEQLGG	26.0	12.7	24.7	0.5	1.9		

FIG. 1M-1

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Clone Design	Sequence	Comparisons			
		E-Tag	IGF ₁ R	IR	IGFR/IR
H2CBa-3-D11-IR	<u>XXXXXXXXXXXXXX</u> <u>XXXXXXXXXXXXXX</u>	27.8	13.0	24.8	0.5
H2CBa-3-H12-IR	GPRGQRLHDAFYSWFDALRVN	27.4	7.2	12.4	0.6
H2CBa-3-A10-IR	LGTIAVEHFLFYGMFEERQLGG	27.1	13.2	22.3	0.6
H2CBa-3-A5-IR	LGGYCGFNC <u>QFYRWF</u> DNLADR	28.3	16.1	28.1	0.6
H2CBa-3-C4-IR	FSGWADYQS <u>GFYQW</u> FAEELAN	30.7	17.2	29.2	0.6
H2CBa-3-B8-IR	WGPFSVFD <u>ESFYRW</u> FAQASDD	25.6	11.3	18.6	0.6
H2CBa-3-H11-IR	PRNEGVLVHGLFYDW <u>FQRALSG</u>	28.8	14.0	22.4	0.6
H2CBa-3-E10-IR	DEGGAPLDV <u>MFYRW</u> FE <u>QAVRG</u>	27.7	14.3	23.0	0.6
H2CBa-3-C2-IR	QSGNRGSHGA <u>FYSW</u> FRDVLAN	28.4	17.0	26.7	0.6
H2CBa-3-F6-IR	MR <u>QRDGFNS</u> SEFYGWFAAALGE	27.3	14.5	21.8	0.7
H2CBa-3-D4-IR	SEERKKVHS <u>QFYSW</u> FDRLQLLG	29.0	18.9	27.1	0.7
H2CBa-3-A7-IR	PSPNAPFH <u>GGFYDW</u> FDWVQGS	29.1	19.4	26.9	0.7
H2CBa-3-H4-IR	FHRPGSFNTNFYQW <u>FDDQM</u> NQ	27.2	20.1	27.9	0.7
H2CBa-3-B7-IR	SDDSSTLN <u>GRFTW</u> FHMQLLD	28.6	18.0	23.6	0.8
H2CBa-3-F9-IR	QRGGGFHE <u>GYSW</u> FRSQSLL	26.1	19.1	24.3	0.8
H2CBa-3-H6-IR	SGSSRPVF <u>HEQFYEW</u> FVDQLGL	24.8	21.6	27.3	0.8
H2CBa-3-F5-IR	GGSSQAFHG <u>GAFYEW</u> FSA <u>QLRG</u>	29.4	22.0	27.8	0.8
H2CBa-3-A2-IR	AFVSERVN <u>QRFYDW</u> FRDQMRS	30.7	22.5	29.1	0.8
H2CBa-3-F3-IR	VRHPTRFHDEF <u>YRW</u> FTEQLLT	16.3	6.7	9.0	0.7
H2CBa-3-G6-IR	ARLLNIFDRG <u>FYNW</u> FQRQLDE	24.9	21.0	24.4	0.9
H2CBa-3-G7-IR	PSLSNLHESFYRW <u>FDQLV</u> ST	24.4	18.7	23.0	0.8
H2CBa-3-C5-IR	FAFGLG <u>FHQGYDW</u> FAHQLEG	26.4	21.2	25.4	0.8
H2CBa-3-G1-IR	VSATV <u>MLHREFYDW</u> FGI <u>QLLD</u>	26.9	21.5	26.3	0.8
H2CBa-3-E3-IR	GGVS <u>GVLHD</u> RFYSW <u>FERQLAG</u>	24.2	17.2	19.3	0.9

FIG. 1M-2

Clone Design	Sequence	Comparisons			
		E-Tag	IGFaR	IR	IR/IGFR
H2CB α -3-A9-IR	XXOXOXFXFXXXXXFXWXXXXXXX	---	30.5	21.7	24.1
H2CB α -3-C11-IR	RVDAALNAGFYEWFRGVIQG	---	26.4	21.8	23.2
H2CB α -3-B4-IR	GGAGRSFHDAFYEWFERQMQAG	---	30.9	22.0	24.3
H2CB α -3-F11-IR	EGARQGFHARFYSWFAQQQLAL	---	24.5	22.5	23.9
H2CB α -3-G10-IR	VLLPGVWHGGFYDWFSRQLSS	---	28.3	23.6	27.1
H2CB α -3-D7-IR	GALSDRYNNVVFYDWFREQLLG	---	31.4	23.6	25.3
H2CB α -3-E2-IR	PDSFMSLHQRFYSWFOAQVGT	---	26.8	24.0	25.7
H2CB α -3-B5-IR	RVYKANFHNEFYGMFREQQLLG	---	28.7	25.0	26.4
H2CB α -3-C7-IR	HSGMRDVHARFYSWFSEQLSG	---	30.0	25.2	28.7
H2CB α -3-G9-IR	ARLLERFQDPFYEWFFETLMGD	---	27.8	25.2	26.7
H2CB α -3-A12-IR	RNSSCGNFHDKFYNWFEAQQLKG	---	28.0	26.4	28.7
H2CB α -3-C9-IR	GSMSPVENDQFIGWFRDLVDE	---	32.1	28.7	31.9
H2CB α -3-B10-IR	SCTGRQFDGCFYAWFEDQLVG	---	33.5	30.8	33.2
H2CB α -3-E1-IR	GIAVQSLHDSFYRWFDNALGS	---	31.7	30.5	29.0
H2CB α -3-G12-IR	IGPPGSLHHRGFYDWFAEQVEA	---	29.1	31.4	29.8
H2CB α -3-F7-IR	GVDTDFHKDEFYSWFQRQLNG	---	23.2	20.7	20.3
H2CB α -3-G8-IR	WAGRAGIHGFFYEWFRNRQLRG	---	22.8	20.9	20.4
H2CB α -3-C6-IR	LGQLLAFFHLGFFYEWFSEAVAA	---	26.7	21.2	22.0
H2CB α -3-H9-IR	VHSVSRLNVGFYQWFQDQLSG	---	23.4	22.5	22.0
H2CB α -3-H8-IR	LGIMAIIFDRGGFYGMFEEQQQLSG	---	23.5	23.4	23.2
H2CB α -3-F2-IR	VARGSSLHDDFYEWFASQLRT	---	25.5	24.3	25.2
H2CB α -3-D5-IR	LGXYIGALNTQFYSWFADLVGS	---	26.7	24.5	25.6
H2CB α -3-D10-IR	EDSRRLHEGFYGYWFRKQLGD	---	26.8	24.9	24.9
H2CB α -3-F10-IR	GRDNMKFHSGFYDWFTQQLAG	---	25.7	25.6	26.1

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FIG. 1M-3

Clone Design	Sequence	Comparisons					
		E-Tag	IGF1R	IR	IGFR/IR	IR/IGFR	--
H2CBa-3-D6-IR	XXXXXXXXXX	27.9	26.0	25.8	1.0	1.0	
H2CBa-3-H3-IR	AGVMGGFH <u>QEFYLYWFERALSN</u>	27.0	26.9	26.2	1.0	1.0	
H2CBa-3-F4-IR	AGHVGQVYDGFYGW <u>FREQLGA</u>	31.2	27.2	27.7	1.0	1.0	
H2CBa-3-E9-IR	FVQNIGF <u>DYDFYGW</u> FVREVEK	31.6	27.7	28.2	1.0	1.0	
H2CBa-3-H10-IR	PVGIGGLHRAFYQW <u>FQS</u> QVDA	26.9	27.9	28.8	1.0	1.0	
H2CBa-3-G2-IR	GSR <u>QEADHQAFYDW</u> FLVLGV	29.1	28.1	28.8	1.0	1.0	
H2CBa-3-B2-IR	AGGRKPF <u>HDDFYGWF</u> FRDQLAE	29.4	28.1	28.2	1.0	1.0	
H2CBa-3-E8-IR	DLASHGF <u>HDAFYNWFSV</u> QLNS	31.5	28.4	29.1	1.0	1.0	
H2CBa-3-E5-IR	GSNGGGVH <u>GQFYAWF</u> VEALSG	33.0	28.7	28.9	1.0	1.0	
H2CBa-3-E6-IR	RGRAS <u>TFHDGFYGWFSQ</u> QLRF	29.6	29.0	28.1	1.0	1.0	
H2CBa-3-E7-IR	SPARRVSH <u>HDFYGWFA</u> KQLES	30.4	30.2	30.2	1.0	1.0	
H2CBa-3-C8-IR	SSDVGA <u>FHSAYDWFKAQ</u> LSG	31.9	31.2	31.5	1.0	1.0	
H2CBa-3-A4-IR	PTVHRAFD <u>DLFYGWFA</u> KQVED	32.2	31.9	32.6	1.0	1.0	
H2CBa-3-D1-IR	SSNTVGL <u>DERFYAWFVDQ</u> QLGA	32.9	32.5	31.5	1.0	1.0	
H2CBa-3-B9-IR	PGAAEG <u>FHSAYDWFA</u> QQAVSG	33.2	33.8	33.3	1.0	1.0	
H2CBa-3-D8-IR	MRSEASF <u>HVEFYSWFEE</u> QLRS	26.3	20.2	19.1	1.1	0.9	
H2CBa-3-F1-IR	VSRYGG <u>QQDGFYHWFS</u> DLKG	28.8	28.0	26.4	1.1	0.9	
H2CBa-3-A11-IR	RPSSGG <u>GLHYGFTYHWFRV</u> QEEM	20.5	21.5	17.7	1.2	0.8	
H2CBa-3-A3-IR	SNIEEHFM <u>QFYRWFS</u> DALGN	30.4	29.6	21.8	1.4	0.7	
	ANDC <u>CLGLHAGFYGW</u> FAQ <u>Q</u> GG						27/122

FIG. 1M-4

Clone Design	Sequence	Comparisons					
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
H2CB β -3-E8-IR	XXXXXXXXXXXXXXFXXXXXXFXXXXXXFXXXXXX	--	--	--	--	--	--
H2CB β -4-F8-IR	TGHRLGLD E QFYWWFRDALSG	15.9	1.9	11.8	0.2	6.1	
H2CB β -3-C4-IR	VLTSNTLHQRFYSWFAAARRE	13.4	0.8	2.6	0.3	3.4	
H2CB β -3-D5-IR	CVAQQGFOSSFYCWFAGLDID	21.1	1.3	4.0	0.3	3.1	
H2CB β -3-E6-IR	NGQSSRFHTAFYDWFAAQQLSG	14.0	3.3	10.2	0.3	3.1	
H2CB β -4-G12-IR	SVPRGTVHDAFYQWFREREVALG	5.7	0.7	2.1	0.3	3.1	
H2CB β -4-F4-IR	GARGSTTFHDQFYEWFWVQLGD	6.8	1.8	5.4	0.3	3.1	
H2CB β -4-F11-IR	PPGMNGFHTSFYSWFDQLGD	17.9	1.9	5.6	0.3	3.0	
H2CB β -3-E5-IR	AVGTLGYHSQFYRWFERQLGG	15.0	1.7	4.8	0.3	2.9	
H2CB β -4-F2-IR	ELQARGVHRNFYRWFEAQVSG	17.0	1.8	5.0	0.4	2.8	
H2CB β -4-G4-IR	HRVARAFHEQFYDWFERKAVSG	15.9	1.3	3.4	0.4	2.6	
H2CB β -3-C8-IR	GAMEPDYHRSFYQWFAAALGE	8.7	1.4	3.5	0.4	2.6	
H2CB β -4-F10-IR	CPDRQSVDDRFYNWFDALAS	4.9	1.4	3.2	0.4	2.3	
H2CB β -4-H4-IR	CGAQISFHFRFYQWFLQEAG	10.2	1.0	2.4	0.4	2.3	
H2CB β -4-G6-IR	HKRGIVYQHGAFYAWFDSLSSG	20.8	4.2	9.5	0.4	2.3	
H2CB β -4-H1-IR	QASDNRSRDGQFYLWFEKLSS	14.5	5.6	8.5	0.7	1.5	
	DRGRMVGVDGFYNNWFAQM E Q	17.0	10.1	13.2	0.8	1.3	
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FIG. 1M-5

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Clone Design	Sequence	Comparisons					
		E-Tag	Background	IGFsR	IR	IGFR/IR	IR/IGFR
H2CB-3-D2-IGFR	XXXXXXXXXXFYXWFXXXXXXX	--	29.8	17.5	16.3	1.1	0.9
H2CB-3-C12-IGFR	VTFSAV <u>FHENF</u> YDWFVTRQVS		22.9	18.6	11.8	1.6	0.6
H2CB-3-B11-IGFR	TAS <u>QEC</u> FDGGFYGWFRRAWRCT		17.3	19.6	13.0	1.5	0.7
H2CB-4-E2-IGFR	SLDWWRWSEEP <u>FYRW</u> FQRALAG		24.6	17.1	11.9	1.4	0.7
H2CB-3-A5-IGFR	CMSLSDCCHRKFYGWFKS <u>QGGE</u>		22.4	21.0	16.5	1.3	0.8
H2CB-4-G12-IGFR	IALLCRSPGS <u>FYGW</u> FQA <u>AVGC</u>		28.8	26.1	22.6	1.2	0.9
H2CB-3-B2-IGFR	PRSATMSDGGFYWW <u>FASQLGL</u>		23.7	23.8	19.4	1.2	0.8
H2CB-3-D1-IGFR	LRRSSVFHDPPFYE*ISRLVGG		23.0	19.9	16.4	1.2	0.8
H2CB-3-B6-IGFR	ARLQQQFHGGGYEW <u>FRAQVSP</u>		21.5	19.5	15.7	1.2	0.8
H2CB-4-F7-IGFR	AQLDNLCHEPFYSWFCAVTRE		16.3	4.5	3.7	1.2	0.8
H2CB-4-G8-IGFR	WTCDTAFHQDFYQW <u>FCDKLGV</u>		22.0	19.0	18.0	1.1	0.9
H2CB-3-D4-IGFR	GKEFGFLDRD <u>FYWW</u> FR <u>EQQLGP</u>		20.2	18.6	16.5	1.1	0.9
H2CB-3-D5-IGFR	GRAPSSFD <u>CDFYCW</u> FRN <u>IQVQS</u>		21.9	18.3	16.9	1.1	0.9
H2CB-4-E6-IGFR	DVEAET <u>QHRL</u> FYAW <u>FLSQLGS</u>		21.4	17.9	16.4	1.1	0.9
H2CB-3-C2-IGFR	ISVTAVFH <u>DGFY</u> GW <u>FNEBQVSK</u>		19.6	15.8	14.8	1.1	0.9
H2CB-3-A6-IGFR	NSEHGRLL <u>DVDFY</u> GW <u>FARV1QQ</u>		18.8	12.2	10.8	1.1	0.9
H2CB-4-H12-IGFR	GPLGDGC <u>QDG</u> FYGW <u>FMCQVST</u>		26.8	29.0	28.1	1.0	1.0
H2CB-3-B10-IGFR	KRSAYNFHDP <u>FYDW</u> FR <u>MQLSG</u>		23.9	28.3	28.1	1.0	1.0
H2CB-4-F11-IGFR	ASEPGGYLD <u>DPFY</u> GW <u>FR<u>EQOLRA</u></u>		27.1	27.5	27.3	1.0	1.0
H2CB-4-G11-IGFR	NRGDDGGVHS <u>G</u> FYNW <u>FRILQLSG</u>		25.5	25.5	24.6	1.0	1.0
H2CB-4-E12-IGFR	ASKGSSLHND <u>DFY</u> GW <u>FAQQLAR</u>		25.3	25.4	25.3	1.0	1.0
H2CB-4-G10-IGFR	ANVSMWIQVG <u>FYDW</u> DA <u>QLRQ</u>		27.8	24.9	24.7	1.0	1.0
H2CB-3-B9-IGFR	RTSPGSLHDP <u>FYDW</u> F <u>QQQLGG</u>		25.1	24.6	24.2	1.0	1.0
H2CB-3-B7-IGFR	PGVMSSFHG <u>GFY</u> SW <u>FR<u>EQQLNG</u></u>		25.6	23.3	23.7	1.0	1.0
H2CB-4-H4-IGFR	CLANSED <u>HDSF</u> Y <u>GWFCQALGG</u>		24.0	23.2	23.5	1.0	1.0
	GGSMGGMHGS <u>FYEW</u> FA <u>QLRS</u>		23.5	23.1	23.8	1.0	1.0
	RP <u>QGGS</u> I <u>HAGF</u> YQW <u>FRDAVAG</u>						

FIG. 1N-1

Clone Design	Sequence	Comparisons			
		E-Tag	Ratios over Background	IGFR/IR	IR/IGFR
H2CB-4-H10-IGFR	XXXXXXXXXX	--	21.9	22.4	23.3
H2CB-4-H5-IGFR	GALSLSLEDAAFYDWFNRLQLEG	--	22.3	22.3	1.0
H2CB-4-G7-IGFR	KVDLRGFDHDFGYGWFAFQLAG	--	23.1	21.6	1.0
H2CB-4-F4-IGFR	CSGLQRCHDSFSYWSWESVVRE	--	21.3	20.6	1.0
H2CB-3-D8-IGFR	DSLGISFHEGFDYDWFRRLQDM	--	21.3	20.9	1.0
H2CB-4-E4-IGFR	SGVFNGTFYDWFRRIQLGE	--	20.0	20.5	1.0
H2CB-4-E5-IGFR	GYREMRSDLGFYQWFRDQLGLI	--	21.6	20.5	1.0
H2CB-4-E8-IGFR	SVFMQDHVGFYAWFRSLMEE	--	22.0	19.9	1.0
H2CB-3-D12-IGFR	FRHITEVDRRSFYGMWFEVEQLRG	--	21.1	19.7	1.1
H2CB-4-G9-IGFR	WAGGSDDVGSFYDWFRQLIAS	--	26.6	17.3	1.1
H2CB-3-C8-IGFR	GLQNVSFHSGFYEWFRQVSQ	--	21.6	14.5	1.0
H2CB-3-A12-IGFR	SRVSDPYHVGFYQWFEEVVRG	--	20.8	13.4	1.0
H2CB-3-B12-IGFR	MGGATFFHTGFYDWFAAQLQH	--	28.6	27.5	1.0
H2CB-3-A9-IGFR	RPASRPFHSGFYQWFAADQLSH	--	27.8	25.2	1.0
H2CB-3-A3-IGFR	GLAPGNFHEDFYRWFQEQTLG	--	27.7	24.3	1.0
H2CB-3-B4-IGFR	TAAISDFNSLFLFYGMWFEQLISS	--	26.9	24.1	1.0
H2CB-4-E7-IGFR	LDEDLPQHAGFYGMWFAEALGV	--	25.8	23.8	1.0
H2CB-4-G6-IGFR	ASHKSAFDNNFYRWFMSMQLRD	--	24.6	21.6	1.0
H2CB-4-E9-IGFR	HTGAGDLHGAFYNWFLEQLGG	--	22.4	21.1	1.0
H2CB-4-H2-IGFR	RRGRDGFHGGFYDWFAAQLSD	--	24.3	20.7	1.0
H2CB-3-A10-IGFR	GNFREAFHADFSWFERQLQS	--	21.6	20.2	1.0
H2CB-3-C4-IGFR	RDTLPAPHQHFFYQWFEKQVSA	--	24.3	19.9	1.0
H2CB-3-B5-IGFR	ERETAAFGQQAFYQWFRDQIAG	--	23.1	19.2	1.0
H2CB-4-G4-IGFR	WGEGGGFYDWFYDQLGWEPSPH	--	24.2	18.8	1.0
H2CB-3-D9-IGFR	SLVAADLHEGFYGMFRSQLGG	--	21.7	18.7	1.0
H2CB-3-C3-IGFR	TSEVGDFHAEFYSWFEIQLGR	--	24.4	18.6	1.0
H2CB-3-D3-IGFR	TGADCLLHARFYAWFEQOLRE	--	20.3	18.4	1.0
H2CB-4-F2-IGFR	RRDSSLHRSFYDWFSVQLLN	--	22.5	18.3	1.0
	SESKYLLHSGGFYGMWFEAQLRG	--	18.0	16.8	1.0

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FIG. 1N-2

Clone Design	Sequence	Comparisons				
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
H2CB-4-H1-IGFR	<u>XXXXXXXXXXFXXFXXWXXXXXX</u>	--	18.3	15.3	16.5	1.1
H2CB-4-F9-IGFR	HGVIRADHTGFFYAWFEEQVSK	--	22.9	14.4	15.3	1.1
H2CB-4-E10-IGFR	LINA.VFRRGFYAWFEEQVSK	--	26.1	20.1	24.5	1.2
H2CB-4-F8-IGFR	L <u>Q</u> RYIGFHDPFYDWFSRALS	--	21.5	14.8	19.0	1.3
H2CB-3-A8-IGFR	MRTAELFHVGFYDWFDQALMD	--	20.7	14.7	18.2	1.2
H2CB-4-F1-IGFR	WAPPDALHGOFYWRWFQRQLDQ	--	22.2	14.6	18.8	1.3
H2CB-3-C6-IGFR	AHAAATFHDDFYRWFEQVVG	--	15.7	7.8	10.2	0.8
H2CB-4-E11-IGFR	FDAVHGFDGGFYGWFKRELQR	--	26.1	17.6	24.1	0.7
H2CB-3-D6-IGFR	QAGGMEFHGAFYNWFLQQQLSG	--	21.6	13.0	18.8	0.7
H2CB-4-F3-IGFR	GRSVSRMNAEFTQWFGHQLAA	--	17.3	11.1	16.4	0.7
H2CB-3-A4-IGFR	AAVNLSLFDEFYIWLWFDQQLDG	--	27.4	11.0	14.8	0.7
H2CB-3-B1-IGFR	QLGMDWFHADFYEWFLAQQLPS	--	20.0	11.0	15.2	0.7
H2CB-3-C5-IGFR	RLAGSGIHEGFYGWFWFDQLLA	--	19.9	10.5	15.6	0.7
H2CB-4-F6-IGFR	GREIGGVHDGFYDWFRQSEQ	--	18.6	10.1	14.6	0.7
H2CB-3-B8-IGFR	VRSEQRFDSSTFYQWFNDLMS	--	20.7	6.9	9.5	0.7
H2CB-3-C7-IGFR	QSPYGGFFHDGYFWRFLQQTGM	--	16.2	1.8	2.5	0.7
H2CB-4-H7-IGFR	FQCGAAFHVDFYRWFTCQEKF	--	21.8	14.1	22.7	0.6
H2CB-4-F5-IGFR	GAFGSEFHEQFYRWFDALSF	--	12.9	4.0	7.2	0.6
H2CB-4-G1-IGFR	EHTSYQIHRQFYEWFDRALGR	--	20.4	10.3	19.7	0.5
H2CB-3-D11-IGFR	SGTAIDLHSRFYGWFAHQARE	--	24.1	8.8	18.6	0.5
H2CB-3-D7-IGFR	EGFCVLFHGQFYRWFWQLQDG	--	22.1	6.5	13.6	0.5
H2CB-3-C10-IGFR	QQSAGHPHSSSFYLWFSSELLGA	--	21.7	5.1	10.4	0.5
H2CB-4-E3-IGFR	YLQRAGFHRSFYGWFDQALRD	--	20.3	4.6	8.9	0.5
H2CB-3-C1-IGFR	MWILWATLHSDFYSWFEQVVS	--	22.3	6.7	15.7	0.4
H2CB-4-G2-IGFR	GANALGFKDRFYEWFAAQQLWD	--	19.9	3.3	10.7	0.3
H2CB-3-A11-IGFR	GSGLYYVFWHGFFYDWFEQQMGG	--	23.9	2.5	7.7	0.3
H2CB-4-G5-IGFR	LDKGWGFDFLQFYRWFEAATRA	--	19.3	2.5	7.9	0.3
H2CB-4-F12-IGFR	QRSAYEFHADFYDWFLRLTP	--	16.7	1.7	5.4	0.3
	DORMGSFHGEFYRWFEETLLS	--				

FIG. 1N-3

Clone Design	Sequence $X_n-FxWxWx-x_n$	Ratios over Background				Comparisons		
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR		
20E2A-3-B11-IR	GRFYGWFQDAIDQLMPWGFDP	--	24.6	1.4	23.6	0.1	16.8	--
20E2B β -3-E3-IR	IQGWEPPFYGWFDDVVVAQMFE	--	23.0	0.9	15.3	0.1	16.3	--
rB6-3-F6-IR	RYGRWGLAAQQFYDWFDR	--	40.9	1.0	13.3	0.1	13.3	--
RB6-4-F9-IR	RGRLLGSLSLTQFYNWFAE	--	34.1	1.0	12.6	0.1	12.6	--
20E2B α -3-A8-IR	ASAYTPFYQWFADVSEXYMQQ	--	35.4	7.4	34.4	0.2	4.6	--
A6L-4-F6-IR	PYRMEGTEKWNFYDWFAQLQ	--	28.9	4.1	18.1	0.2	4.4	--
20E2B α -4-H9-IR	SAVHFQFYKWFNDNLPPVPLSA	--	37.8	9.4	26.7	0.4	2.9	--
20E2B α -3-B1-IR	VVNKSFYRWFQLVLGSSDDW	--	41.8	12.9	36.8	0.4	2.9	--
20E2B β -4-F9-IR	QSPRASFYGWFDDVLRAAGVV	--	25.9	4.2	10.1	0.4	2.4	--
20E2B β -3-E9-IR	TGFYEWFWYEQIHSRMLPNPLD	--	27.0	7.7	17.2	0.5	2.2	--
20E2B β -3-E10-IR	RRGVGGFYGWFSSQQLQGMGV	--	22.2	2.6	5.5	0.5	2.1	--
20E2B α -3-C12-IR	SSQDERRFYRWFQEAIVGGRDG	--	39.0	6.7	12.0	0.6	1.8	--
20E2B β -3-C12-IR	TRGQLGFLYNWFFQQALSTSMSG	--	20.2	2.2	3.8	0.6	1.8	--
20E2B β -3-E7-IR	CADLNAYFQWFCGVLDRGSDH	--	9.2	1.2	1.9	0.6	1.6	--
20E2B β -3-E11-IR	TLIQDQFYWWFSDLLSAEPGD	--	20.7	1.3	2.1	0.6	1.6	--
20E2B α -3-B11-IR	IDQLDAFYRWFDPGVMLGMDP	--	36.0	20.7	32.8	0.6	1.6	--
NNKH-4-G2-IR	RGGGTFYEWFE SALRKHGAG	--	10.8	6.3	8.9	0.7	1.4	--
20E2B α -3-A7-IR	RGLDQDFYRWFQNLVGVEYDR	--	19.0	4.2	5.5	0.8	1.3	--
20E2B α -4-G12-IR	MQGHRCGYGWFARVLEQDRGW	--	37.0	22.3	29.5	0.8	1.3	--
20E2B α -3-C11-IR	ERLHLRFYEWFDVTIGQDGSD	--	37.3	26.8	34.8	0.8	1.3	--
20E2B α -3-C10-IR	MHVQSDFYHWFQSLLGQQGP	--	37.7	24.8	30.5	0.8	1.2	--

FIG. 1O-1

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Clone Design	Sequence $X_n-FXXWF-X_n$	Ratios over Background				Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/GFR		
20E2Ba-3-D7-IR	TMGTQGFYRWFQNVVKEHLSG	35.4	26.9	31.3	0.9	1.2	—	—
20E2Ba-3-A12-IR	I THN RGFY SWFL DVVQ GAGA	31.7	22.0	23.3	0.9	1.1	—	—
20E2Ba-3-D10-IR	VRR DAGF YQW FAD ILT QLD FE	32.7	27.3	29.1	0.9	1.1	—	—
20E2Ba-4-G7-IR	MQL QD E FY NWF RG IM LND GQ D	34.2	29.0	30.7	0.9	1.1	—	—
20E2Ba-4-F5-IR	GIR SS G F Y Q W F D R V L A G V G D G	33.8	32.1	34.0	0.9	1.1	—	—
20E2Ba-3-C9-IR	A N L N S Q F Y S W F A S V T G E A S P S	39.4	33.2	35.5	0.9	1.1	—	—
20E2Ba-3-A4-IR	QSP R A S F Y G W F D D V L R A G V V	38.2	31.6	35.9	0.9	1.1	—	—
20E2Ba-4-E12-IR	MQR N Q G F Y S W F D D L V S S T V G V	36.0	30.8	29.7	1.0	1.0	—	—
20E2Ba-4-E11-IR	A S G F D P F Y A W F L E Q L R V A N G S	35.1	31.2	30.7	1.0	1.0	—	—
20E2Ba-4-E8-IR	S G T P Y G F Y R W F Q S A L A S A T S G	36.1	30.5	30.7	1.0	1.0	—	—
20E2Ba-4-H10-IR	Q G V E G G F Y E W F D R A M G D V R P W	38.9	30.6	30.7	1.0	1.0	—	—
20E2Ba-4-F6-IR	D N M S G G F Y R W F A Q V V A D S G G D	34.9	33.2	32.0	1.0	1.0	—	—
20E2Ba-4-G4-IR	R G T D D T F Y G W F D Q L L Q G W C D D	34.1	33.7	32.2	1.0	1.0	—	—
20E2Ba-4-F8-IR	T V D H T Q F Y D W F S R V L G E S G S A	37.7	32.0	32.7	1.0	1.0	—	—
20E2Ba-4-G5-IR	G R Q D R E F Y W F T E L Q A G G M D G D	34.9	33.9	33.4	1.0	1.0	—	—
20E2Ba-3-B10-IR	P L L I G G F Y E W F D Q V L K E T K E V	38.2	34.9	33.6	1.0	1.0	—	—
20E2Ba-3-C7-IR	G V L S T G F Y E W F A L Q L H G L A A G	37.6	34.2	34.8	1.0	1.0	—	—
20E2Ba-3-C5-IR	P A V G Q S F Y G W F E A V L R G S K A G	40.4	36.0	35.6	1.0	1.0	—	—
20E2Ba-3-B9-IR	S N G I S G F Y E W F A A Q V O T S D F Q	39.6	35.8	37.1	1.0	1.0	—	—
A6L-4-F11-IR	L L G L S Q A A Y A N F Y D W F V S Q L A	33.1	4.6	4.6	1.0	1.0	—	—
20E2Ba-3-C2-IR	V P N S W M F Y N W F A E Q I E G G E G E	44.1	40.0	38.1	1.0	1.0	—	—
20E2Ba-3-B2-IR	A R R A D G F Y D W F R E Q V S G A V Q	43.1	40.1	39.0	1.0	1.0	—	—
20E2Ba-4-G2-IR	G V V E G T F Y E W F D R I L G G V Q G D	34.1	33.6	29.8	1.1	0.9	—	—
20E2Ba-4-H6-IR	S H L T D P F Y Q W F V D Q L R A G V R G	39.4	36.0	31.9	1.1	0.9	—	—

FIG. 10-2

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Clone Design	Sequence X_n -Fy _n WF-X _m	Comparisons				
		E-Tag	IGF3R	IR	IGFR/IR	IR/IGFR
20E2Ba-4-H5-IR	RSNDDDAFYRWFSNILQVDDGGG	--	--	--	--	--
20E2Ba-4-G3-IR	DSDGAQFYIWFEQDQLRSAAGWD	38.7	35.1	32.3	1.1	0.9
20E2Ba-4-H4-IR	PGLHRAFYQWFAEAIVRSANKE	35.5	36.1	32.7	1.1	0.9
20E2Ba-3-C1-IR	SLGQQGGFYDWFAASOVGGAIDI	38.8	37.9	35.0	1.1	0.9
20E2Ba-4-E6-IR	CGQTQSFYQWFCCEVMRVESGD	43.7	42.1	39.0	1.1	0.9
H5-3-D5-IR	IVVPGGDTQGVNFYDWFWVQLQ	38.0	34.3	29.7	1.2	0.9
JBA5-3-D9-IR	RDVSMGSASTNFYDWFWVQLG	43.8	21.8	18.2	1.2	0.8
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTVHSA	38.3	29.8	25.3	1.2	0.8
20E2Bβ-4-H10-IR	SNGISGFYEWFAAQVQTSDFQ	22.4	6.2	1.9	3.3	0.3
rB6-4-G8-IR	RRDRGGGLDVFFFYQWFMD	23.5	32.2	9.7	3.3	0.3
		--	--	--	--	--

FIG. 10-3

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Clone Design	Sequence	Comparisons		
		IGFR/R	IR	IGFR/IR
R20β-4-F8-IR	HLCVLEELFWGASLFGYCSG	39.1	1.8	27.7

FIG. 2A

Clone Design	Sequence	Ratios over Background				Comparisons			
		E-Tag	IGFαR	IR	IGFR/R	IGFR/I	IR/GFR	IR/GFR	
F815-4-H9-IR	<u>HLCVLEELFWGASLFGYXCSG</u>	34.9	0.9	<0.1	37.6	<0.1	40.8	--	--
F815-3-B1-IR	PLCVLEELFWSTPLFGQ _Q C SY	31.7	0.9	<0.1	35.8	<0.1	39.3	--	--
F815-3-D1-IR	<u>HLCVLEELFWGASRFQ_QCG</u>	30.4	0.9	<0.1	33.5	<0.1	38.9	--	--
F815-3-D4-IR	DLCVLEELFWGASLFQ _Q CG	31.5	0.9	<0.1	33.6	<0.1	38.8	--	--
F815-3-C5-IR	<u>HLCVVEELFWGASLFQ_QCG</u>	31.1	0.8	<0.1	31.2	<0.1	38.5	--	--
F815-4-H3-IR	NLCDLEVLFWGASLFQ _Q CG	33.7	1.0	<0.1	37.2	<0.1	38.4	--	--
F815-3-A5-IR	PLCVLEEQFWGASLFQ _Q CG	37.4	1.1	<0.1	40.9	<0.1	38.3	--	--
F815-3-D7-IR	<u>QLCVLEELFWGASEFQ_QCG</u>	33.6	0.9	<0.1	34.3	<0.1	38.3	--	--
F815-3-A1-IR	HLCELEELFWGASLFQ _Q CG	29.8	0.9	<0.1	34.8	<0.1	38.0	--	--
F815-4-H4-IR	PLCVLEELFWGESLFQ _Q CG	31.1	0.9	<0.1	32.7	<0.1	38.0	--	--
F815-3-A3-IR	<u>HLCVLEELFWGASRFQ_QCG</u>	32.8	1.0	<0.1	39.1	<0.1	37.9	--	--
F815-3-B3-IR	KLCVLEELFWGASLFQ _Q CG	33.7	1.0	<0.1	37.5	<0.1	37.5	--	--
F815-3-A4-IR	YLCVLEELSWGASLFQ _Q CG	32.5	1.0	<0.1	36.9	<0.1	37.5	--	--
F815-3-D2-IR	<u>HLCVLEELLWGASLFQ_QCG</u>	31.9	0.9	<0.1	34.1	<0.1	37.4	--	--
F815-3-C4-IR	QLCVLEQFWGESLFQ _Q CG	31.6	0.8	<0.1	31.8	<0.1	37.4	--	--
F815-3-B4-IR	HLCVLEELFWGNLFSQC _Q CG	33.8	1.0	<0.1	36.7	<0.1	37.3	--	--
F815-3-C1-IR	HLCVLEELFWGASLYGQ _Q CG	29.0	0.9	<0.1	35.0	<0.1	37.3	--	--
F815-4-G9-IR	<u>SLCALEEQFWGAALFGYXCSG</u>	36.5	1.0	<0.1	38.9	<0.1	37.1	--	--
F815-4-G6-IR	HLCVLEEEQFWGASLFDGCGAG	34.9	1.0	<0.1	36.4	<0.1	37.0	--	--
F815-3-A8-IR	QLCVLEELFWGASLFQ _Q CG	34.7	1.1	<0.1	39.3	<0.1	36.9	--	--
F815-4-G5-IR	<u>PLCVLEELFWGAALFGQ_QCG</u>	26.5	1.0	<0.1	35.1	<0.1	36.8	--	--
F815-3-B5-IR	HLCVLEELFWGASLFQ _Q CTG	33.2	0.9	<0.1	34.1	<0.1	36.8	--	--
F815-4-F4-IR	PLCVLEELFWGGSLFQ _Q CG	28.6	0.8	<0.1	30.0	<0.1	36.7	--	--
F815-3-A2-IR	<u>QLCVLEELWVGASLFQ_QCSR</u>	32.5	1.0	<0.1	36.6	<0.1	36.6	--	--
F815-3-B6-IR	HLCVVEELIWGASLFQ _Q CSR	31.6	0.9	<0.1	32.9	<0.1	36.5	--	--
F815-4-H7-IR	DLCVLEELFWGASLFQ _Q CG	33.7	1.0	<0.1	37.6	<0.1	36.4	--	--
F815-4-H8-IR	QLCVLEERFWGASLFQ _Q CG	35.8	1.0	<0.1	37.0	<0.1	36.4	--	--
F815-4-G7-IR	<u>NLCVLEELFWGAALFGQ_QCG</u>	33.7	1.0	<0.1	35.8	<0.1	36.3	--	--

FIG. 2B-1

	Clone Design	Sequence	Comparisons			
			E-Tag	IGF1R	IR	IGFR/R
F815-3-A6-IR	<u>HLCVLEELFWGASLFGYCSG</u>	34.6	1.1	39.0	<0.1	36.2
F815-3-D3-IR	<u>QLCVLEELFWGSSLFGQCSG</u>	33.8	1.0	36.2	<0.1	36.2
F815-3-B12-IR	<u>DLCVVEELFWGKSILFGQCSG</u>	33.2	1.0	35.7	<0.1	36.2
F815-4-G10-IR	<u>DLCVLEELFWGSSLFGQCSG</u>	35.4	1.0	37.2	<0.1	36.1
F815-4-E3-IR	<u>YLCVLEEQFWGASLFRQCFG</u>	32.4	1.0	35.0	<0.1	36.1
F815-4-E6-IR	<u>HLCVLEELWGSSLFGQCSG</u>	33.2	1.0	34.5	<0.1	36.1
F815-4-F1-IR	<u>PLCGLEELFWGASLFGQCSD</u>	29.4	0.9	32.5	<0.1	36.0
F815-4-G8-IR	<u>HLCVLEELFWGSSLFAQCSG</u>	36.8	1.1	38.2	<0.1	35.9
F815-4-H12-IR	<u>PLCAIEELFWGAALFGQCSG</u>	30.5	0.9	31.9	<0.1	35.9
F815-4-G3-IR	<u>HLCVLEEQFWGASLFGDCSG</u>	31.4	1.0	35.7	<0.1	35.7
F815-3-C2-IR	<u>DLCGLEELFWGAALFGQCTS</u>	32.3	1.0	36.1	<0.1	35.6
F815-4-E10-IR	<u>QLCVLEKQLWGASLFMOCSG</u>	35.4	1.0	36.5	<0.1	35.4
F815-3-A12-IR	<u>HLCVLEELFWGASLYGQCPG</u>	32.1	1.0	36.3	<0.1	35.3
F815-3-B8-IR	<u>HLCVLEELFWGASLFQDCSG</u>	33.6	1.0	35.8	<0.1	35.3
F815-3-B2-IR	<u>HLCVLEELWGASLFQCSG</u>	31.0	1.0	35.3	<0.1	35.3
F815-3-C3-IR	<u>PLCVLEELFWGVSLFGQCGG</u>	30.1	1.0	35.3	<0.1	35.3
F815-3-A7-IR	<u>HLCVLEELFWGASQWGQCSG</u>	33.1	1.0	35.8	<0.1	35.2
F815-4-F9-IR	<u>RLCVLEEQFWGGALFGQCSG</u>	33.4	1.0	35.7	<0.1	35.2
F815-3-B7-IR	<u>QLCVLEELFWGVSLFAQCSG</u>	32.0	1.0	33.5	<0.1	35.0
F815-4-E4-IR	<u>HLCVLEELFWGAALFGQCFG</u>	28.0	1.0	33.4	<0.1	35.0
F815-4-E12-IR	<u>YLCVLEELFWGASQFGQCSG</u>	28.0	0.9	30.2	<0.1	34.8
F815-4-F8-IR	<u>HLCVLEELYWGASLFQCSG</u>	33.8	1.0	35.2	<0.1	34.7
F815-3-C7-IR	<u>HLCVLEERFWGVSLFGQCSG</u>	33.9	1.0	34.7	<0.1	34.7
F815-4-F10-IR	<u>PLCVLEELFWGASRFQCSG</u>	32.7	1.0	34.2	<0.1	34.7
F815-3-D11-IR	<u>HLCVLEDLFWGASLFQCSG</u>	35.4	1.1	37.3	<0.1	34.6
F815-4-E7-IR	<u>HLCDILELFWGASLFGQCSG</u>	30.3	0.9	32.2	<0.1	34.6
F815-3-A10-IR	<u>QLCILEEQFWGTSLFGYCSG</u>	34.0	1.1	36.4	<0.1	34.3
F815-3-B11-IR	<u>ALCVLEELFWGEESLFQCSG</u>	33.7	1.1	36.3	<0.1	34.2

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FIG. 2B-2

Clone Design	Sequence	Comparisons			
		E-Tag	Background	IGF ₁ R	IR
F815-4-F11-IR	<u>HLCVLEELFWGASLFGYCSG</u>	--	31.8	1.0	<0.1
F815-4-A9-IR	<u>RLCVLEERFWGAALFGQ₂CSCG</u>	31.9	1.0	33.7	34.2
F815-4-G11-IR	<u>PLCVLEELFWGASLFGQ₂CSCG</u>	31.9	1.0	<0.1	<0.1
F815-3-D8-IR	<u>SLCVLEELFWGGSRFQGQ₂CSCG</u>	32.3	1.0	34.4	34.1
F815-4-G4-IR	<u>HLCLLEEQFWGASLFGQ₂CSCG</u>	32.3	1.0	<0.1	33.9
F815-3-C8-IR	<u>HLCVLEEQFWGASLFGQ₂CSCG</u>	23.8	1.0	32.2	<0.1
F815-4-G12-IR	<u>DLCLEEELMWGASRFQGQ₂CSCG</u>	33.9	1.0	35.1	33.7
F815-3-D12-IR	<u>YLCVLEERFWGASLFGQ₂CSCG</u>	31.7	1.0	33.5	33.5
F815-4-F7-IR	<u>HLCVLLEEQLFWGASLFGQ₂CSCG</u>	33.3	1.0	34.8	<0.1
F815-4-F2-IR	<u>QLCVLLEEQLWGASLFGQ₂CSCG</u>	26.1	1.0	<0.1	33.4
F815-3-B9-IR	<u>HLCVLEELF*GESLFGYCSG</u>	33.6	1.1	35.7	<0.1
F815-4-H2-IR	<u>PLCVLEELFWGASHFGQ₂CSCG</u>	36.1	1.2	38.4	<0.1
F815-4-E11-IR	<u>HLCVLEELVWGASLFGQ₂CAG</u>	33.2	1.1	35.4	<0.1
F815-4-G1-IR	<u>QLCVLEELVWGASLFGQ₂CAG</u>	27.9	1.0	31.5	<0.1
F815-3-A11-IR	<u>HLCGLEELFWGASLFGQ₂CSCG</u>	37.7	1.2	40.1	<0.1
F815-4-F6-IR	<u>HLCVLEELVWGESLFGQ₂CSCG</u>	32.3	1.1	34.6	<0.1
F815-3-D9-IR	<u>RLCVLEELYWGASLFGQ₂CSCG</u>	31.4	1.0	32.5	<0.1
F815-3-C11-IR	<u>RLCILEELFWGASLFGQ₂CSCG</u>	33.4	1.1	35.7	<0.1
F815-4-G2-IR	<u>HLCVLEELFWGATLFQ₂CSCG</u>	30.2	1.1	34.3	<0.1
F815-3-C9-IR	<u>HLCFEEELFWGASMFGQ₂CSCG</u>	29.7	1.0	31.4	<0.1
F815-4-H10-IR	<u>HLCLIVEELFWAAPLFQ₂CSCG</u>	31.9	0.9	27.6	29.4
F815-4-F3-IR	<u>HLCVLEELVWGASLFA₂QCSA</u>	19.4	1.0	28.0	<0.1
F815-4-F5-IR	<u>NLCALEELFWGASQFRYCPG</u>	12.3	0.9	24.8	26.8
F815-4-H1-IR	<u>RLCVLEELFWGASLFGQ₂CSCG</u>	6.9	1.0	15.8	16.5
F815-4-E5-IR	<u>PLCVLEELFWGASLFGQ₂CPG</u>	3.5	1.0	13.6	0.1
F815-4-H5-IR	<u>NLCVLEELFWGASLFGQ₂CSCG</u>	5.5	1.0	13.1	0.1
F815-3-C10-IR	<u>QLCVLG#RFWGGSLCGYCS</u>	3.5	1.1	5.2	4.5

FIG. 2B-3

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Clone	Parental/Design	Comparisons			
		E-Tag	IGF.R	IR	IGFR/IR
F815-4-F11-IGFR	HLCVLEELFWGASLFGYXCSG	39.1	1.8	27.7	0.1 15.4
F815-4-E12-IGFR	PLCFLQELFFGASLGGYCSG	33.4	12.3	1.0	12.3 0.1
F815-4-H10-IGFR	FMCGGLQELVGGAALLGHCSG	33.7	15.1	1.7	8.9 0.1
F815-4-B7-IGFR	PLCFLQELFGGGSLSGYCSG	30.1	8.5	1.0	8.5 0.1
F815-3-B5-IGFR	FLCGGLEELAWGVSRSGYCFG	35.2	23.9	4.8	5.0 0.2
F815-4-D12-IGFR	PLCFLAELFSGSALGGDCSR	33.9	4.8	1.0	4.8 0.2
F815-4-C11-IGFR	PLCVLQELFGGGSLGGYCSG	33.6	7.0	1.8	3.9 0.3
F815-4-C7-IGFR	QLCVL#LFWMGACLFGYCAG	13.9	4.6	1.8	2.6 0.4
F815-4-E7-IGFR	FLCGGLQELSGVASLFGQCSG	16.8	2.0	1.0	2.0 0.5
F815-4-G7-IGFR	RVCVLEQLVWGASLFGA*SG	26.9	3.8	1.9	2.0 0.5
F815-4-A10-IGFR	FYCGGLEELSWGAAALFGYCSG	30.4	9.0	5.0	1.8 0.6
F815-3-B3-IGFR	FLCGGLEELSQQGAVLFHCYG	30.8	3.7	2.2	1.7 0.6
F815-3-G1-IGFR	HLCVLVGLFWDASLFGQCSG	7.6	1.0	2.0	0.5 2.0
F815-4-G12-IGFR	QRClRAALFWCATLLGGCAG	20.5	1.0	2.0	0.5 2.0
F815-3-H1-IGFR	HQCIPDGMSQQGAALRGNCSD	7.6	1.0	2.5	0.4 2.5
	HLCVLEDELWGVSLFGYCSS	18.4	1.0	6.8	0.1 6.8

FIG. 2C

Clone	Parental/Design	Comparisons					
		IGFR/IR	IGF1R	IGFR/IR	IGF1R	IR	IGFR/IR
F820-4-B5-IR		39.1	1.8	27.7	0.1	15.4	
F820-4-A2-IR		28.1	0.9	17.9	<0.1	21.1	
F820-4-E2-IR		34.0	1.6	22.7	0.1	13.9	
F820-4-D10-IR		21.3	0.7	9.0	0.1	13.4	
F820-4-H7-IR		34.1	1.0	12.1	0.1	12.1	
F820-4-G6-IR		14.2	0.6	6.5	0.1	11.6	
F820-4-C2-IR		14.0	0.5	6.1	0.1	11.5	
F820-4-B4-IR		38.1	1.2	11.8	0.1	9.9	
F820-4-C7-IR		15.1	0.7	6.4	0.1	8.7	
F820-4-F10-IR		46.3	2.7	22.2	0.1	8.2	
F820-4-G5-IR		14.5	0.6	4.7	0.1	8.0	
F820-4-F2-IR		8.8	0.6	4.4	0.1	7.5	
F820-4-H8-IR		11.7	0.6	4.2	0.1	7.4	
F820-4-D7-IR		16.0	0.6	4.7	0.1	7.3	
F820-4-B2-IR		14.5	0.8	5.9	0.1	7.1	
F820-4-C3-IR		5.0	0.4	2.4	0.2	6.9	
F820-4-H4-IR		37.5	1.1	7.5	0.2	6.6	
F820-4-B10-IR		21.2	1.1	6.4	0.2	5.9	
F820-4-A5-IR		7.5	0.7	3.7	0.2	5.6	
F820-4-F6-IR		5.3	0.8	4.4	0.2	5.2	
F820-4-F1-IR		5.7	0.6	3.0	0.2	4.9	
F820-4-A3-IR		9.1	1.0	4.7	0.2	4.7	
F820-4-D1-IR		5.9	0.8	3.5	0.2	4.5	
F820-4-F5-IR		25.7	2.3	10.5	0.2	4.5	
F820-4-F12-IR		3.7	0.6	2.7	0.2	4.2	
F820-4-A11-IR		11.3	0.6	2.2	0.3	3.5	
F820-4-E8-IR		7.6	0.8	2.7	0.3	3.5	
F820-4-H3-IR		8.0	1.2	4.3	0.3	3.4	
		17.5	2.6	9.0	0.3		

FIG. 2D-1

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	Clone Parental/Design	Sequence	Comparisons					
			IGFR/IR	IR	IGFR/IR	IR	IGFR/IR	IR
			--	--	--	--	--	--
F820-4-A8-IR	<u>HLCVLEELFWGASLFGTCG</u>	QLCVMEELFWGASRFQCGSG	6.4	0.7	2.4	0.3	3.4	
F820-4-G1-IR		HLCVLEELFWGASMFGQCGSG	3.9	0.6	1.9	0.3	3.4	
F820-4-F3-IR		QLCVLEMFWGGSRFVQCSA	9.8	1.3	3.6	0.4	2.9	
F820-4-D6-IR		PLCILEELFWGEALFDQCGA	5.4	1.2	3.2	0.4	2.6	
F820-4-A1-IR		YLCVQEELFWGASLFGYCIV	25.5	2.4	6.1	0.4	2.5	
F820-4-H2-IR		HLCALEEAFGPSSLFNSCQG	15.9	1.6	4.1	0.4	2.5	
F820-4-F4-IR		HLCVLEERFWGASLFGQCGSG	6.8	1.9	4.7	0.4	2.5	
F820-4-B6-IR		OLCDLEELFWGASLFGYCPG	4.1	0.8	1.9	0.4	2.4	
F820-4-B11-IR		HLCVLEERFWGASIWGCSCG	22.2	3.1	7.0	0.4	2.3	
F820-4-H6-IR		QLCVLEELFWGGSLWGQCSR	4.1	1.1	2.4	0.5	2.2	
F820-4-H9-IR		PLCVLEELFWGAAQFGQCGS	3.1	0.9	1.9	0.5	2.1	
F820-4-D3-IR		OLCDLEERFWGVSLFGLCSCG	4.6	1.3	2.5	0.5	1.9	
F820-4-C1-IR		QLCVLEEVFWGASLFGLCTG	13.0	1.1	2.1	0.5	1.9	
F820-4-D12-IR		QL.DLNNTWSGLCLCSVTVRV	10.4	1.2	2.0	0.6	1.7	
F820-4-B8-IR		DLCVLEESLWGKALFGYCSD	7.2	2.2	3.4	0.6	1.5	
F820-4-C6-IR		HLCVLEEVFWGSSMFGDCSG	13.9	2.5	2.8	0.9	1.1	
F820-4-C10-IR		HLCDLEELFWGASLFGDCQG	5.3	2.6	2.9	0.9	1.1	
F820-4-D4-IR		QLCVLDALMWGGCRUGHQCG	3.5	2.3	2.1	1.1	0.9	
F820-4-E1-IR		OLCVLEEKFWGTSILFGDCMG	1.6	1.6	1.5	1.1	0.9	
F820-4-B3-IR		QLCVLEEVFWGAQFGSCSG	15.9	0.6	5.0	1.2	0.8	
F820-4-D2-IR		QLCVLEELFWGPSMFGYCSCG	7.8	3.2	2.5	1.3	0.8	
F820-4-C5-IR		HLCDLEELFWGASGFACCYG	21.5	4.0	2.3	1.8	0.6	

FIG. 2D-2

Clone Design	Sequence	Comparisons			
		E-Tag	IGF1R	IR	IGFR/IR
A6L-3-C4-IR	<u>HLCVLEELFWGASLFGYCSG</u>	--	36.9	1.0	40.5 <0.1 42.5
A6L-3-D7-IR	DLCVLEERFWGASLFGQCSG	--	38.6	1.0	40.1 <0.1 40.7
A6L-3-A1-IR	QLCVLEELHWGASLFGYCSCG	--	39.6	1.1	44.8 <0.1 40.6
A6L-3-C1-IR	PLCVLEEQFWGASLFGQCSG	--	37.3	1.0	40.3 <0.1 40.3
A6L-3-D5-IR	YLCDLEERFWGASLFGQCSS	--	42.9	1.1	44.4 <0.1 40.2
A6L-3-A4-IR	HLCLLEERFWGSSQFGFCSCG	--	26.7	1.1	42.2 <0.1 40.2
A6L-3-D3-IR	HLCVLEELFWGASQFGQCSG	--	34.6	0.9	36.9 <0.1 39.8
A6L-3-B1-IR	HLCVLEERFWGASLFGQCTG	--	33.9	1.0	38.7 <0.1 39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSG	--	35.3	1.1	42.4 <0.1 38.6
A6L-3-B2-IR	HLCVLEERFWGASLFSQCSG	--	38.1	1.1	42.7 <0.1 37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSG	--	31.6	1.1	39.6 <0.1 36.7
B6C-4-H10-IR	QLCLLEELFWGASLFGQCSG	--	38.5	1.1	41.1 <0.1 36.5
B6H-4-GB-IR	HLCVLEEMFWGASLFGQCSG	--	31.7	1.1	39.7 <0.1 36.2
A6L-3-D6-IR	HLCDLLEELFWGASLFSQCSR	--	35.5	1.0	37.2 <0.1 36.1
B6C-4-F1-IR	QLCVLEELFWGASLFGYCSG	--	32.9	1.1	38.7 <0.1 35.8
B6C-4-H3-IR	QLCALLEQFWGASLFSQCSG	--	37.4	1.2	40.5 <0.1 34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSG	--	30.2	1.0	35.7 <0.1 34.3
B6C-4-G1-IR	HLCVLEEWFWGDSLFGQCSR	--	34.9	1.2	40.2 <0.1 33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSG	--	34.4	1.2	38.8 <0.1 33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSG	--	34.7	1.2	39.6 <0.1 32.8
B6C-4-F11-IR	HLCVLEELFWGASRFQGQCSG	--	34.0	1.2	37.2 <0.1 31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSA	--	32.3	1.2	37.4 <0.1 30.6
B6C-4-E12-IR	HLCVLEELIWGASRFQGQCSG	--	30.9	1.1	33.3 <0.1 30.2
B6C-4-G10-IR	HLCVLEELFWGGSLFIQCSG	--	33.0	1.3	40.3 <0.1 30.1
B6C-4-F8-IR	QLCVLEEEQFWGASLFGNCSC	--	36.4	1.4	39.8 <0.1 29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSG	--	26.6	1.1	32.5 <0.1 29.2
B6C-4-G3-IR	HLCVLEEMFWGASLFGQCGG	--	34.0	1.4	38.8 <0.1 28.3
20C-3-B7-IR	PLCVLEELVWGASLFGVQCSG	--	29.5	1.2	32.9 <0.1 28.3

FIG. 2E-1

Clone Design	Sequence	Comparisons			
		E-Tag	Ratios over Background	IGF1R	IR
		--	--	--	--
20C-3-B4-IR	<u>HLCVLEELFWGASLFGYCSG</u>	28.9	1.1	<0.1	28.0
20C-3-C11-IR	NLCVLEELFWGESLFGQCSG	30.2	1.1	<0.1	27.7
B6C-4-G2-IR	HLCVLE <u>QFWGGSLFGYCSR</u>	29.4	1.3	35.3	27.5
20C-3-B8-IR	HLCFILEEVFWGA <u>LFQACSG</u>	28.5	1.1	31.2	27.4
20C-3-C10-IR	HLCFILEVLFWGSAL <u>FGQCSG</u>	32.1	1.2	33.6	27.1
20C-3-B6-IR	HLCVLEERFWGASLFW <u>QCSG</u>	29.7	1.2	31.9	26.7
A6L-3-A3-IR	HLCVLEEQYWGE <u>SLFGYCSG</u>	14.4	1.1	28.3	26.5
A6L-3-B3-IR	PLCVLIE <u>QFWGASLFAYCSS</u>	38.7	1.7	43.4	26.3
20C-3-A5-IR	QLCVLEELFWGESLFA <u>QCLG</u>	22.9	1.1	27.6	26.0
20C-3-B11-IR	HLCVLEELFWGQ <u>SLFGHCSD</u>	30.0	1.3	32.7	25.8
20C-3-B3-IR	HLCVLEELVW <u>GASLFGFCSG</u>	29.3	1.2	31.2	25.7
20C-3-C12-IR	LLCVLIEEQFWG <u>ASLFGQCSG</u>	29.6	1.3	31.8	24.8
20C-3-C3-IR	RLCVLEELFWG <u>ESLFGQCSG</u>	30.1	1.2	30.1	24.3
20C-3-C2-IR	HLCVLEEMFWG <u>ASLFGNCSG</u>	29.9	1.3	29.8	23.8
20C-3-A11-IR	ELCFLEELFWG <u>ASLFGQCSG</u>	25.9	1.2	27.4	23.0
20C-3-A4-IR	HLCVLEELFWGASL <u>LYGQCSS</u>	27.2	1.2	27.5	22.9
20C-3-A6-IR	HLCVLEELFWG <u>ASLFAQCPG</u>	26.1	1.2	27.5	22.8
B6C-4-E4-IR	NLCVLEELFWG <u>ASEFGQCSG</u>	34.5	1.7	39.1	22.7
20C-3-A9-IR	DLCVLEEQLWG <u>ASLFRYCSG</u>	29.7	1.3	29.3	22.7
B6C-3-C5-IR	HLCVLE <u>QFWGVALFGNCSG</u>	33.5	1.7	37.7	22.5
20C-3-B1-IR	HLCVLE <u>QIWGASLFGQCSG</u>	30.2	1.2	26.7	22.0
20C-3-A10-IR	HLCVLEERFWGG <u>ALFGQCTA</u>	29.0	1.3	28.5	21.5
20C-4-F1-IR	HLCFILEELFWG <u>ASLFGQCSG</u>	29.1	1.4	29.5	20.7
20C-4-E1-IR	QLCGLEELFWG <u>ASLFGYCSA</u>	28.3	1.4	29.7	20.6
20C-3-B12-IR	QLCGLEELFWG <u>ASLFGQCSS</u>	27.0	1.3	25.8	20.2
20C-3-A8-IR	HLCVLEELYWG <u>ASQFGQCSG</u>	21.1	1.1	21.2	20.0
20C-3-A7-IR	FLCVLEELYWG <u>ASQFGYCSG</u>	21.9	1.3	23.0	18.3
B6C-4-E10-IR	HLCVLE <u>QFWGASLFGYCSG</u>	35.2	2.2	38.0	17.5

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Sequence

HLCVLEELFWGASLFGYCSG

NLCVLEELFWGESLFGQCSG

HLCVLEQFWGGSLFGYCSR

HLCFILEEVFWGALFQACSG

HLCFILEVLFWGSALFGQCSG

HLCVMEELFWGASLFGQCSG

HLCVLEERFWGASLFWQCSG

HLCVLEEQYWGESLFGYCSG

PLCVLIEQFWGASLFAYCSS

QLCVLEELFWGESLFAQCLG

HLCVLEELFWGQSLFGHCSD

HLCVLEELVWGASLFGFCSG

LLCVLIEEQFWGASLFGQCSG

RLCVLEELFWGESLFGQCSG

HLCVLEEMFWGASLFGNCSG

ELCFLEELFWGASLFGQCSG

HLCVLEELFWGASLLYGQCSS

HLCVLEELFWGASLFAQCPG

NLCVLEELFWGASEFGQCSG

DLCVLEEQLWGASLFRYCSG

HLCVLEQFWGVALFGNCSG

HLCVLEQIWGASLFGQCSG

HLCVLEERFWGGALFGQCTA

HLCFILEELFWGASLFGQCSG

QLCGLEELFWGASLFGYCSA

QLCGLEELFWGASLFGQCSS

HLCVLEELYWGASQFGQCSG

FLCVLEELYWGASQFGYCSG

HLCVLEQFWGASLFGYCSG

FIG. 2E-2

Clone Design	Sequence	Comparisons			
		E-Tag	IGFsr	IR	IGFR/IR
20C-3-A1-IR	<u>HLCVLEELFWGASLFGYCSG</u>	--	21.0	1.1	17.6
20C-3-C1-IR	<u>RLCALEELFWGASLFGQCSG</u>		30.6	1.4	21.9
A6L-3-D2-IR	<u>HLCVLEEQFWGASLFGQCSG</u>		7.0	1.1	14.9
B6C-4-G12-IR	<u>QLCVLEELFWGSSRLGYCSG</u>		31.1	2.5	33.5
B6H-4-F9-IR	<u>DLCVLEELFWGASLFGQCSG</u>		39.3	3.6	43.1
B6C-4-E3-IR	<u>QLCLIEEQFWWGSLFGQCSG</u>		34.6	5.3	40.0
20C-3-B10-IR	<u>HLCVLEELFWGTSLFGQCSG</u>		29.9	16.9	31.7
20C-3-A3-IR	<u>RLCVLEELFWGASLFDQCSR</u>		28.4	19.1	25.3

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FIG. 2E-3

Clone Design	Sequence
20C-3-A1-IR	<u>HLCVLEELFWGASLFGYCSG</u>
20C-3-C1-IR	<u>RLCALEELFWGASLFGQCSG</u>
A6L-3-D2-IR	<u>HLCVLEEQFWGASLFGQCSG</u>
B6C-4-G12-IR	<u>QLCVLEELFWGSSRLGYCSG</u>
B6H-4-F9-IR	<u>DLCVLEELFWGASLFGQCSG</u>
B6C-4-E3-IR	<u>QLCLIEEQFWWGSLFGQCSG</u>
20C-3-B10-IR	<u>HLCVLEELFWGTSLFGQCSG</u>
20C-3-A3-IR	<u>RLCVLEELFWGASLFDQCSR</u>

Clone Design	Sequence	Ratios over Background				Comparisons	
		L-Tag	IGF ₁ R	IR	---	IGFR/IR	IGFR
R20 α -3-20A4-IR	XXXXXX	--	--	--	--	0.1	14.4
R20 β -4-A7-IR	EIEAEWGRVRCVCLVYGRCVGG	50.2	1.6	23.1	0.1	18.5	45/122
R20 β -4-DB-IR	EIEAEWGRVRCVCLVYGRCVGG	44.2	1.3	24.0	0.1	17.3	
	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1		

FIG. 3A

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Clone Parental/Design	Sequence	Comparisons			
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D815-4-A8-IR	<u>WLDQEWAWVQCEVYGRGCP</u> <u>WLDLEWAQVQCEVYGRGCP</u>	44.8	1.4	24.2	<0.1 17.3
D815-4-D10-IR	<u>WLDQEWAWVQCEVYGRGCP</u>	48.0	1.0	48.4	<0.1 48.4
D815-4-D9-IR	<u>WLDQEWAWVQCEVYGRGCP</u> <u>WLDQEWQQVQCQVYGRGCTS</u>	49.2	1.0	48.2	<0.1 48.2
D815-4-A11-IR	<u>RLDEEWARVQCEVYGRGCRS</u>	47.5	1.0	48.0	<0.1 48.0
D815-4-E12-IR	<u>WLEQEWAWIQCQVYGRGCP</u>	47.9	1.0	48.0	<0.1 48.0
D815-4-B7-IR	<u>WLEQEWAWVQCEVYGRGCP</u>	49.0	1.0	47.6	<0.1 47.6
D815-4-D11-IR	<u>WLDEEWIQCCKVYGRGCPA</u>	45.4	1.0	47.2	<0.1 47.2
D815-4-D12-IR	<u>WLEQEWAWVQCEVYGRGCP</u>	49.5	1.0	47.0	<0.1 47.0
D815-4-F8-IR	<u>WLDQEWAWIQCQVYGRGCPA</u>	48.1	1.0	46.6	<0.1 46.6
D815-4-A9-IR	<u>SLDWEAWLQCQVYGRGCP</u>	47.8	1.0	46.4	<0.1 46.4
D815-4-E9-IR	<u>WLEQEWQVRCLVYGRGCPP</u>	47.7	1.0	45.8	<0.1 45.8
D815-4-B10-IR	<u>WLDQEWAWVQCEVYGRGCPY</u>	49.0	1.0	45.6	<0.1 45.6
D815-4-H8-IR	<u>WLDQEWAGVLCEVYGRGCP</u>	49.0	1.0	45.6	<0.1 45.6
D815-4-E10-IR	<u>SLDKIEWEWVLCCVYGRGCP</u>	47.0	1.0	45.6	<0.1 45.6
D815-4-D7-IR	<u>WLEQEWAWVQCEVYGRGCRS</u>	44.5	1.0	45.4	<0.1 45.4
D815-4-G9-IR	<u>WLEEWEWAQVQCAVYGRGCSS</u>	44.2	1.0	44.2	<0.1 44.2
D815-4-G12-IR	<u>WLDQEWALVQCEVYGRGCP</u>	44.3	1.0	43.7	<0.1 43.7
D815-4-E11-IR	<u>WLDQEWAWVQCEVYGRGCP</u>	45.5	1.0	43.0	<0.1 43.0
D815-4-H7-IR	<u>WLEQEWAWVQCEVYGRGCAS</u>	46.2	1.0	43.0	<0.1 43.0
D815-4-F12-IR	<u>WLDQEWAWVEVYGRRCPS</u>	47.2	1.0	42.6	<0.1 42.6
D815-4-E8-IR	<u>WLDQEWAWVQCEVYGRGCP</u>	47.9	1.0	42.6	<0.1 42.6
D815-4-F9-IR	<u>QLDQEWAWVLCKVYGRGCP</u>	46.4	1.0	41.8	<0.1 41.8
D815-4-A10-IR	<u>WLDHE*AWVQCEVYGRGCP</u>	47.3	1.0	41.2	<0.1 41.2
D815-4-C7-IR	<u>QLEQEWAWVRCQVYGRGCSS</u>	37.7	1.0	40.0	<0.1 40.0
D815-4-H10-IR	<u>WLDQEWAWVQCEVYGRGCLS</u>	47.0	1.0	39.8	<0.1 39.8
D815-4-C9-IR	<u>WLDQEWAWVRCQVYGLGCP</u>	44.2	1.0	39.8	<0.1 39.8
D815-4-F11-IR	<u>WLDQEWAWVMKCELYGRGCP</u>	40.4	1.0	39.2	<0.1 39.2
D815-4-H12-IR	<u>WLEQEWAWVQCEVYGRGCLS</u>	45.4	1.0	38.6	<0.1 38.6
D815-4-A7-IR	<u>SLDQEWAWVQCEVYGRGCLS</u>	37.3	1.0	37.3	<0.1 37.3
D815-4-H11-IR	<u>WLDHEAWAWVQCEVYGRGCTS</u>	2.4	1.0	37.2	<0.1 37.2
D815-4-F7-IR	<u>WLDVEWAWVQCEVYGRGCP</u>	32.4	1.0	34.7	<0.1 34.7

FIG. 3B-1

Clone Parental/Design	Sequence	Comparisons			
		E-Tag	IGF ₁ R	IR	IGFR/IR IR/IGFR
D815-4-G8-IR	<u>WLDQEWAWVQCEVYGRGCPS</u>	--	--	--	--
D815-4-G7-IR	OLDQEWAVRVC E VWGRGCSS	27.8	1.0	33.6	<0.1 33.6
D815-4-G11-IR	WLDLEWA <u>Q</u> VQCKVYGRGCPS	34.7	1.0	32.3	<0.1 32.3
D815-4-E7-IR	WLDEEWAWVQC <u>Q</u> VYGRGCPS	30.7	1.0	28.6	<0.1 28.6
D815-4-A12-IR	WLDQEWAWVQCEVWGRGCAF	33.0	1.0	26.4	<0.1 26.4
D815-4-B11-IR	WLDREWA <u>Q</u> VQCEVYGRGCLS	28.4	1.0	19.0	0.1 19.0
D815-4-D8-IR	SLDREWAYVQC <u>Q</u> VYGRGCSS	20.8	1.0	18.8	0.1 18.8
				14.6	0.1 14.6

FIG. 3B-2

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Clone Parental/Design	Sequence	Comparisons			
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D820-3-H2-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.8	1.4	24.2	0.1 17.2
D820-3-C4-IR	RDLLEWANIQCEVYGRGCP	23.9	1.0	40.0	<0.1 40.0
D820-3-C3-IR	WLEQEWAWVQCEVYGRGCS	31.0	1.0	39.5	<0.1 39.5
D820-3-G6-IR	WLEQE W ILVECEVYGRGCP	35.2	1.0	39.4	<0.1 39.4
D820-3-D2-IR	WLDQEWENW I QCEVYGRGCP	35.6	1.0	38.8	<0.1 38.8
D820-3-B3-IR	LLDEEWAQIECEIYGRGCP	34.8	1.0	37.7	<0.1 37.7
D820-3-B5-IR	ALEEEWA W VQCEVYGRGCHF	34.1	1.0	37.1	<0.1 37.1
D820-3-E2-IR	C?EQEWGLYQCEVYGRGCP	34.4	1.0	37.0	<0.1 37.0
D820-3-B3-IR	WLEQEWAWVQCEVYGRGCP	33.6	1.0	36.7	<0.1 36.7
D820-3-B6-IR	WLEHEWA Q VQCEVWGRGCPY	31.2	1.0	36.6	<0.1 36.6
D820-3-D4-IR	WLEQEWAAEVRC E VYGRGCP	32.0	1.0	36.2	<0.1 36.2
D820-3-C2-IR	?LEQEWAWVQCEVYGRGCP	33.7	1.0	35.6	<0.1 35.6
D820-3-F6-IR	WLEQEWAGIQCKVYGRGCP	30.8	1.0	35.2	<0.1 35.2
D820-3-D5-IR	RLEQEWAAQVQCEVWGRGCLP	30.5	1.0	34.8	<0.1 34.8
D820-3-F5-IR	OLDHEWAGIQCEVWGRGCP	29.8	1.0	34.6	<0.1 34.6
D820-3-H3-IR	WLEQEWAAQI Q CEVYGAGCRS	30.2	1.0	33.8	<0.1 33.8
D820-3-G2-IR	SLEQEWAWVQC V VYGRGCP	31.3	1.0	33.0	<0.1 33.0
D820-3-H6-IR	WLEQEWWDQVLCEVYGRGCP	30.3	1.0	32.2	<0.1 32.2
D820-3-F3-IR	WLEQEWAAQV?CEVYGRGCA?	28.6	1.0	30.7	<0.1 30.7
D820-3-B4-IR	WMDQEWAWVQCEVYGRGCP	33.1	1.0	30.5	<0.1 30.5
D820-3-C5-IR	QLDQEWAWI Q CEVYGRNCRT	29.1	1.0	30.3	<0.1 30.3
D820-3-F4-IR	TLEQEWAAQVIC E VYGRGCLS	25.9	1.0	29.5	<0.1 29.5
D820-3-H5-IR	RLEQEWAWVQCEVWGRGCLS	26.3	1.0	28.6	<0.1 28.6
D820-3-A6-IR	WLDQEWAAQIQC H WGRGCPA	24.8	1.0	26.0	<0.1 26.0
D820-3-A2-IR	WLDQEWAAQIQC H WGRGCPA	23.7	1.0	25.6	<0.1 25.6
D820-3-G5-IR	WLEQEWAWVQCEVYGRGCP	22.6	1.0	25.0	<0.1 25.0
D820-3-G3-IR	RLEEEWA W VQC Q VYGRGCP	22.2	1.0	23.9	<0.1 23.9
D820-3-E3-IR	WLEQEWVRIQCEVYGRGCP	20.6	1.0	22.7	<0.1 22.7

FIG. 3C-1

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	Clone Parental/Design	Sequence	Comparisons			
			E-Tag	IGFsR	IR	IGFR/IR
		<u>WLDQEWAWVQCEVYGRGCP</u> S	44.8	1.4	24.2	0.1
		WLEQEWTVQCEVYGGCPS	25.9	1.0	22.6	<0.1
		WLEKEWAGVQCEIYGRGCP	27.3	1.0	22.4	<0.1
		WLEEWEAWVRCEVYGRGCQS	22.4	1.0	21.9	<0.1
		WLEHEWA <u>Q</u> IQCCELYGRGCTY	22.0	1.0	21.0	<0.1
		ALEEEWEAWVQCEVYGRGCP	13.1	1.0	18.4	0.1
		WLEQEWAWQCEVYGRGCP	23.5	1.0	18.4	0.1
		WLDDEWA <u>Q</u> IQCCEIYGRGCS	25.6	1.0	17.5	0.1
		QLEEEWAGVQCEVYGRECP	14.5	1.0	16.3	0.1
		WLEQEWLLVQCGVYGRGCP	27.8	1.0	13.9	0.1
		WLDQEWA <u>W</u> IQCCEVYGRGCRS	14.7	1.0	12.8	0.1
		WLEQEWAWQCEVSGRGCP	6.4	1.0	6.3	0.2
		W?DQEWA <u>L</u> IQCCEVYGRGCP	13.7	1.0	6.2	0.2
		SLDEEWAGVLCEVYGRGCPF	6.0	1.0	4.3	0.2
		SVDQELEWLIMCHFQGRVCP	34.9	9.0	10.9	0.8
		WLEQERAWIWCEI <u>Q</u> GSGCRA	32.2	8.6	1.0	8.6
						0.1

FIG. 3C-2

Clone	Parental/Design	Sequence
D820-3-E5-IR		<u>WLDQEWAWVQCEVYGRGCP</u> S
D820-3-D1-IR		WLEQEWTVQCEVYGGCPS
D820-3-E1-IR		WLEKEWAGVQCEIYGRGCP
D820-3-F1-IR		WLEEWEAWVRCEVYGRGCQS
D820-3-B2-IR		WLEHEWA <u>Q</u> IQCCELYGRGCTY
D820-3-A3-IR		ALEEEWEAWVQCEVYGRGCP
D820-3-H4-IR		WLEQEWAWQCEVYGRGCP
D820-3-G1-IR		WLDDEWA <u>Q</u> IQCCEIYGRGCS
D820-3-C1-IR		QLEEEWAGVQCEVYGRECP
D820-3-A1-IR		WLEQEWLLVQCGVYGRGCP
D820-3-A5-IR		WLDQEWA <u>W</u> IQCCEVYGRGCRS
D820-3-H1-IR		WLEQEWAWQCEVSGRGCP
D820-3-A4-IR		W?DQEWA <u>L</u> IQCCEVYGRGCP
D820-4-E12-IR		SLDEEWAGVLCEVYGRGCPF
D820-4-B12-IR		SVDQELEWLIMCHFQGRVCP
		WLEQERAWIWCEI <u>Q</u> GSGCRA

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	Clone	Parental/Design	Comparisons			
			E-Tag	IGFaR	IR	IGFR/IR
	D820-3-D5-IGFR		44.8	1.4	24.2	17.3
	D820-3-E4-IGFR		29.6	3.8	1.0	3.8
	D820-3-C5-IGFR		27.1	3.2	1.0	3.2
	D820-3-F4-IGFR		25.0	3.1	1.0	3.1
	D820-3-F6-IGFR		3.9	2.7	1.0	2.7
	D820-3-G4-IGFR		26.7	2.6	1.0	2.6
	D820-3-E2-IGFR		3.8	2.6	1.0	2.6
	D820-3-G6-IGFR		15.3	2.6	1.0	2.6
	D820-4-A11-IGFR		20.3	2.4	1.0	2.4
	D820-4-H11-IGFR		12.6	1.0	3.0	0.3
	D820-4-C8-IGFR		8.1	1.0	4.6	0.2
	D820-4-D11-IGFR		4.5	1.0	5.3	0.2
	D820-4-A8-IGFR		3.2	1.0	5.5	0.2
	D820-4-F9-IGFR		9.4	1.0	5.8	0.2
	D820-4-D7-IGFR		11.6	1.0	5.9	0.2
	D820-4-D9-IGFR		10.1	1.0	8.9	0.1
	D820-4-H9-IGFR		34.1	3.5	33.4	0.1
	D820-4-E10-IGFR		18.4	1.0	10.1	0.1
	D820-4-E7-IGFR		26.7	2.6	27.0	0.1
	D820-4-H8-IGFR		31.9	3.0	32.2	0.1
	D820-4-A11-IGFR		16.1	1.0	11.7	0.1
	D820-4-C9-IGFR		8.0	1.0	12.5	0.1
	D820-4-E9-IGFR		19.6	1.0	14.9	0.1
	D820-4-B10-IGFR		19.3	1.0	17.3	0.1
	D820-4-F10-IGFR		19.3	1.0	21.5	<0.1
	D820-4-B9-IGFR		27.8	1.0	25.7	<0.1
	D820-4-G8-IGFR		31.7	1.0	26.5	<0.1
			25.6	1.0	29.3	<0.1
						29.3

Sequence
<u>WLDQEWAWVQCEVYGRGCP</u>
WVNQALGGVQS DVQGRRCQS
LLDHEWPWVGC EVCGRGSLS
WLHQELAWVRGEYPRGRRS
WLGHDWAWIQC E VYGLGCPC
WIDQEGRVYQCEA *GRAFPS
WRDEEWAWVQGVYQGRGWPA
RLGVIEWSWFOR KVYGRDSTS
WLAQGNAGVQCVVYGRGCRN
WLEEE *AGIQCQV?GRGCP
WLDQEWEWVQCEVWGRGCLS
RLEQEWA L I Q C E V Y G R G C P S
WLEEEWAQVQCVQVYGRGCAS
WLDLIE *E WLQCEVYGRGCAT
WLEQE W V Q V R C E V Y G R G C P S
WLEEEWAQVQCEVYGRGCPS
WLDQEWA R V Q C E V W G R G C T Y
YLD?EWA W V Q C E V Y G L G Q S
WLDVE *A W V Q C E V W G R G C P S
WLEQEWER?QCEVYGRGCPP
WLEEEWAQVQCEVYGRGCLS
WLDQEWA W I Q C E V Y G R G C P S
?LEHEWAQIQC E V ?GRGQS
WL?QEWA W I Q C E V Y G R G C P F
WLD?EWA W V Q C E V Y G R G C P S
GLEQGCPRWVGLEVQCRGGCP
WLEEEWAQVQCEVYGHGCPs
WLDQEWAQIQC E V Y G R G C S S

FIG. 3D-1

Clone Parental/Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGF _a R	IR	IGFR/IR	IR/IGFR		
D820-4-G9-IGFR	<u>WLDQEWAWVQCEVYGRGCP\$</u>	44.8	1.4	24.2	0.1	17.3		
D820-4-C10-IGFR	WLDQEWAWVQCEVWGRGCP\$	36.8	1.0	29.6	<0.1	29.6		
D820-4-A9-IGFR	WLDLWEFVQCEVYGRGCP\$	32.6	1.0	31.3	<0.1	31.3		
D820-4-B8-IGFR	WLEQEWASVQCEVYGRGCP\$	20.4	1.0	31.4	<0.1	31.4		
D820-4-F8-IGFR	WLDLWEQIKCKVYGRGCP\$	31.1	1.0	32.7	<0.1	32.7		
D820-4-H7-IGFR	WLEQEWAIQCCIQYGRGCP\$	28.3	1.0	32.9	<0.1	32.9		
D820-4-E8-IGFR	WLEQEWALVLCCEVYGHGCC\$	34.1	1.0	32.9	<0.1	32.9		
D820-4-G10-IGFR	WLEQEWAIQCEVWGRGCS\$	26.6	1.0	33.2	<0.1	33.2		
D820-4-D10-IGFR	WLEQEWAIQCEVYGRGC\$S	37.5	1.0	33.2	<0.1	33.2		
D820-4-D8-IGFR	WLEQEWAWAQVQCDVYGRGCP\$	36.6	1.0	33.5	<0.1	33.5		
D820-4-A10-IGFR	WLEQE*ARVQCEVWGRGCP\$	23.7	1.0	34.6	<0.1	34.6		
D820-4-B7-IGFR	WL?QEWARVHCEVWGRP?QC	29.4	1.0	35.5	<0.1	35.5		
D820-4-E12-IGFR	PLEHEWAWVQCVVYGRGCR\$	35.4	1.0	36.9	<0.1	36.9		
D820-4-H10-IGFR	SLE?EWAWVQCEV?GRGCP?	37.0	1.0	37.0	<0.1	37.0		
D820-4-F12-IGFR	WLDQEWVRVQCEVWGRGCP\$	36.8	1.0	37.1	<0.1	37.1		
D820-4-F7-IGFR	SLDKIEWAWVKCEVYGRGCP\$	36.9	1.0	37.3	<0.1	37.3		
D820-4-G12-IGFR	LGDQEWAWVMEV#GRGPWS	34.4	1.0	37.5	<0.1	37.5		
D820-4-D12-IGFR	WLEEIEWAQIRCQVYGRGCP\$	30.3	1.0	37.8	<0.1	37.8		
D820-4-A12-IGFR	WLEE*GWVQCEVWGRGCP\$	37.2	1.0	38.6	<0.1	38.6		
D820-4-C12-IGFR	CLDQEWA?VQCPVYGRGCP\$	30.4	1.0	39.3	<0.1	39.3		
D820-4-A7-IGFR	QLELEWARVQCEVWDRGCP\$	37.1	1.0	39.6	<0.1	39.6		
D820-4-B12-IGFR	RLEQEWAWIQCCEVYGRGCR\$	35.4	1.0	40.8	<0.1	40.8		
	SLEHE*AWVQCKVYGRGC\$S	36.2	1.0	41.4	<0.1	41.4		

FIG. 3D-2
FIG. 3E

Clone Parental/Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGF _a R	IR	IGFR/IR	IR/IGFR		
B6-4-G12-IR	<u>WLDQEWAWVQCEVYGRGCP\$</u>	44.8	1.4	24.2	<0.1	17.3		
B6-3-A11-IR	WLDQEWAWIQCCEVYGRGCP\$	4.4	1.0	6.9	0.1	7.1		
	WLDQEWAWVRCCEVYGRGCP\$	7.3	1.0	6.3	0.2	6.3		

Clone Design
R20-4-C10-IGFR

Clone Design	Comparisons		
	E-Tag	IGFsr	IR
R20-4-C10-IGFR	--	--	--

FIG. 4A-1

Clone Design	Sequence	Comparisons
20F-4-B7-IGFR	TPIAGGINIASWGGTYWLS	IGFR/IR IR/IGFR
20F-4-E4-IGFR	HRGTVTGVWVARWPGYEWLS	--
20F-4-E12-IGFR	SDWAQPQRNRNDWPGYHWL	52/122
20F-4-F4-IGFR	SDWAQPQRNRNDWPGYHWL	0.1
20F-4-F7-IGFR	HRGTVTGVWVARWPGYEWLS	0.2
20F-4-E7-IGFR	SDWAQPQRNRNDWPGYHWL	0.2
20F-4-F11-IGFR	RPHRRINPQDDAVWPGYLWLG	0.2
20F-4-D10-IGFR	HRGTVTGVWVARWPGYEWLS	0.2
20F-4-B3-IGFR	FGRGYGGDGGGWSGYEWLA	0.2
20F-4-B12-IGFR	DGLVVKSGREWPGYEWLER.A	0.2
20F-3-A9-IGFR	DGSIV.VSSSVGMPGYEWLM	0.2
20F-4-G2-IGFR	WQQANLSNGGGGRWGGYDWLM	0.2
20F-4-D11-IGFR	FGRGYGGDGGGWSGYEWLA	0.4
20F-4-G4-IGFR	VNYEMDRVPMPWGGYWWLS	0.4
20F-4-G12-IGFR	MGGGLMWVGVH1WPGYSWLSQ	0.6

Clone Design	Comparisons		
	E-Tag	IGFsr	IR
R20-4-C10-IGFR	--	--	--

FIG. 4A-2

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
R20 β -4-A4-IR	WPGYLFFEEALQDWRGSTD	--	--	--	--	--	--
R20 β -4-F2-IR	SMFVAGSDRWPGYGVLA DWL	11.9	17.5	1.4	12.5	0.1	
R20 β -4-E8-IR	VRGFQQGTWPGYEWLRNAA	16.4	13.9	3.1	4.5	0.2	
		41.0	34.9	3.6	9.7	0.1	

FIG. 4B-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
20F-4-H10-IR	LDLASGDSWLGYDVLRGWLS	--	--	--	--	--	53/122
20F-4-C10-IR	IHSSSDGIGAWGGYAWFRDVA	10.2	3.1	2.4	1.3	0.8	
		23.4	9.6	4.1	2.3	0.4	

FIG. 4B-2

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
R20 β -4-D10-IR	YDKKKKKKKKKKKKKKKKK	--	--	--	--	--	
R20 β -4-D9b-IR	LGPLLRLWGSEVCGVWPDLCE	21.5	1.0	8.0	0.1	8.0	
R20 β -4-H4-IR	PFGFGGRWWGIPRMWWYRNS	32.6	6.8	15.1	0.5	2.2	
R20 β -4-A2-IR	WWGGCRNRNWLERWGLGER	11.6	1.7	3.6	0.5	2.1	
		17.1	2.6	5.2	0.5	2.0	

FIG. 4C

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
R20 β -4-D10-IR	WPGYLFFEEALQDWRGSTD	--	--	--	--	--	
R20 β -4-F2-IR	SMFVAGSDRWPGYGVLA DWL	11.9	17.5	1.4	12.5	0.1	
R20 β -4-E8-IR	VRGFQQGTWPGYEWLRNAA	16.4	13.9	3.1	4.5	0.2	
		41.0	34.9	3.6	9.7	0.1	

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
R20 β -4-H4-IR	YDKKKKKKKKKKKKKKKKK	--	--	--	--	--	
R20 β -4-A2-IR	LGPLLRLWGSEVCGVWPDLCE	21.5	1.0	8.0	0.1	8.0	
R20 β -4-C10-IR	PFGFGGRWWGIPRMWWYRNS	32.6	6.8	15.1	0.5	2.2	
R20 β -4-D9b-IR	WWGGCRNRNWLERWGLGER	11.6	1.7	3.6	0.5	2.1	
		17.1	2.6	5.2	0.5	2.0	

FIG. 4C

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Clone Design	Sequence	Comparisons			
		E-Tag	IGFsR	IR	IGFR/IR
--	--	--	--	--	--
R40-3-40A2-IR	RGTRTDRLWKGFFAIIVPRWPCFSYHCLVEWITKTGSPG	44.6	1.5	2.7	0.6
R40-4-40F10-IR	GRTSMAFVPPRH <u>L</u> QPELA <u>P</u> PRVRNHAWLVG <u>G</u> G	46.4	1.9	2.1	0.9

FIG. 4D

Clone Design	Sequence	Comparisons			
		E-Tag	IGFsR	IR	IGFR/IR
--	--	--	--	--	--
R20-4-F10-IGFR	CLGAGGSFRAGILCLGGGPVS	35.5	6.0	--	--
R20-4-F7-IGFR	GFWATACGGLQICEELGLKP	29.1	4.7	--	--
R20-4-H9-IGFR	DLFCAAMA <u>Q</u> AL <u>G</u> LG <u>Q</u> DLSCG	25.7	3.0	--	--
R20-3-A4-IGFR	RHLLPQIWIAS*GGWGMG	15.6	2.7	--	--

FIG. 4E

Clone Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGFaR	IR	IGFR/IR	IR/IGFR	--	--
20C-3-H3-IGFR	XXXXXXXXXXXXXX	28.5	26.6	1.0	26.6	<0.1		
20C-3-F4-IGFR	DHRLCGTDEYLMDLFLVRLGLCRLLW	34.4	27.5	1.2	23.1	<0.1		
20C-4-C10-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR	35.5	24.4	1.3	19.2	0.1		
20C-3-G5-IGFR	IWIACI D ELLJRGQVWSSCRRAPIG	29.3	21.1	1.1	18.7	0.1		
20C-3-A2-IGFR	DWLRCLGVLISGGLTTELANTGCVQG	33.9	18.3	1.1	16.9	0.1		
20C-3-B4-IGFR	WFSEFCLGGLLQAQEWSMGRDVGCIGY	39.8	29.1	1.9	15.2	0.1		
20C-3-C6-IGFR	GYSWLRDVLMEKMQAQLKREGSVGRQFLTRILLERLGLS*ERGEAGGGPYAQAFSGFCMGLERLSQVSLGCGAGQQG	34.8	20.9	1.4	14.9	0.1		
20C-3-E2-IGFR	ISFRCQLFLVLAGMHPCPVDVGGEGFNTPNCSQDWGOESGFMALLLALTCKLQGFCELLATVTGVTGLGCLDYQPI	34.8	28.1	2.0	14.2	0.1		
20C-3-A3-IGFR	GSSICNLLARAQIVELALCEMGVQE	33.7	14.3	1.2	12.4	0.1		
20C-3-B1-IGFR	L.SFACLLSQLSGVVLPDCLLGEDGEHFQQLMSLCGDDCPVNCGGS	30.2	9.8	0.9	11.2	0.1		
20C-3-F5-IGFR	GWFECLLASLVLQVPQGRSRAAVCYRQECACSVGAVGFLGLACLARSG	35.5	31.9	3.9	8.2	0.1		
20C-4-A7-IGFR	24.7	13.3	2.8	6.9	0.1			
20C-4-F8-IGFR	30.5	27.7	5.3	5.2	0.2			
20C-4-G11-IGFR	34.0	5.1	1.6	3.1	0.3			
20C-3-E1-IGFR	37.3	32.8	13.7	2.4	0.4			
20C-3-B6-IGFR								

FIG. 4F-1

Clone Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGFaR	IR	IGFR/IR	IR/IGFR	--	--
40F-4-D1-IGFR	XXXXXXXXXXXXXX	4.9	4.6	0.3	13.1	0.1		
40F-4-B1-IGFR	LSCLAYSRHGIWRPSTDGLGRSVGEVSSTRWRGYDWFE	4.1	3.0	0.2	13.1	0.1		
40F-4-D10-IGFR	GLDHSDAVGVHLGFAWPA.ARGRWEAGGLEDTWAGYDWLW.GYAWLW	4.9	4.5	0.4	11.7	0.1		
40F-3-A3-IGFR	LSCLAYSRHGIWRPSTDGLGRSVGEVSSTRWRGYDWFE	2.6	2.0	0.3	7.9	0.1		
40F-4-C4-IGFR	EAMAVGLQCPARFVRAAAHGDDGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1	0.2		

FIG. 4F-2

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		Comparisons			
		IGFR/IR	IR/IGFR	IGFR/IR	IR/IGFR
Ratios over Background					
E-Tag	IGF5R	IR	IR	IGFR/IR	IR/IGFR
39.1	1.8	27.7	0.1	15.4	2.1
34.6	7.9	1.0	7.9	0.1	
14.9	1.0	2.0	0.5	2.0	
35.2	1.0	2.0	0.5	2.0	
5.4	1.0	2.1	0.5	2.1	

FIG. 4G

Sequence

HICVYLEELFGASLFGYCSG
HFFVYLVERLGSASLFGSGSA
HFRFREGLLNGAYQFCYCSG
FQSLIIEELVWGAFLFRYGTG
HLSVLEELSWGASLFGWAG

Clone Parental/Design
F815-4-G11-IGFR
F815-3-D1-IGFR
F815-4-C12-IGFR
F815-4-A11-IGFR

Clone Parental/ NNNKH-4-A9
NNNKH-4-H4
NNNKH-4-B3
NNNKH-4-E1
NNNKH-4-E7
NNNKH-4-G3
NNNKH-4-B6
NNNKH-4-A1
NNNKH-4-A5
NNNKH-4-F1
NNNKH-4-C9
NNNKH-4-D1
NNNKH-4-D1
NNNKH-4-E5
NNNKH-2-A6
NNNKH-4-F6
NNNKH-4-C7
NNNKH-4-F7
NNNKH-4-F8
NNNKH-4-E9
NNNKH-4-E6
NNNKH-4-B7
NNNKH-2-B3

Clone Parental/Design	Sequence	Ratios over Background				Comparisons		
		E-Tag	IGFsr	IR	IGFR/IR	IR/IGFR		
NNKH-2-C5-IGFR	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1		
NNKH-2-D9-IGFR	HL*VLEELSWGASLIVGQWAV		7.3	0.9	0.7	1.3	0.8	
NNKH-2-H12-IGFR	HLSVLEEL*LGASMFGGLWAG		4.1	0.5	0.4	1.3	0.8	
NNKH-2-D10-IGFR	HLSVLIKELSW*ASLFGQWAG		5.0	1.3	1.1	1.2	0.8	
NNKH-2-C6-IGFR	HLSALEELSWGASLFGQWAG		4.8	2.1	1.9	1.1	0.9	
NNKH-2-C7-IGFR	HLSVLAELS*GALLFGQWAG		1.9	1.4	1.3	1.1	0.9	
NNKH-2-F11-IGFR	RLSVLEQLSWGASLFGQWAG		18.2	1.0	0.9	1.1	0.9	
NNKH-2-H3-IGFR	HL*VLVQPSWGASLFGQWAG		21.8	1.3	1.3	1.0	1.0	
NNKH-2-B8-IGFR	HQSVLEELSR*ASLFGQWAG		6.7	1.3	1.4	1.4	1.1	
NNKH-2-B12-IGFR	DMSVLLGGLSWGA*LEFGQWSG		4.7	0.7	0.8	0.9	1.1	
NNKH-2-F9-IGFR	HLSVREGOLWRASMFGGRWAG		17.5	3.7	5.2	0.7	1.4	
	QLSVVEL*WGASLFGQWAA			1.2	1.0	2.9	2.9	
	HLSVGEELSW*VALLGQWAR			3.7	0.6	2.1	0.3	3.5

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FIG. 4I

D Name	Clonal Name	Formula	K4(μM) HIGR	Fat Cell Assay	Activity	K4(μM) HIGR	Ratio ICF / IR	Sequence
D101	20D3	1	0.51			1.3	25	KIGGQGAHQDGNYDWVFEALAKK (ε -biotin)
D102	20D1	1	0.27			1.1	41	KVLQARHGCCDSYSDCFYEWFAKK (ε -biotin)
D103	B8	1	0.97			7.4	6.2	
D104	E7	1	0.74			16	16	
D105	H8	1	2.0			15	20	KWSALLSYMIDGYAWFDIAVKK (ε -biotin)
D106	20F1	1	0.97	YES		>20	>1	KGHISWALVRHMDRLFYEWFDLKK (ε -biotin)
D107	40G11	1	1.1			12	4.3	KRKPKTDOEEQNWSFYEWFRHKK (ε -biotin)
D108	JG11	1	2.3					
D109	20H1	1	3.6					
D110	G3	1	0.84					
D111	D2	1	0.62					
D112	IGFR C1	1	0.49					
	A65-4-C1		0.19					
D113	IGFR H2	1	0.75	-20 μM	Agonist	5.4	7.2	DYKDVFTSAVFHENFYDWVYQVSKK (ε -biotin)
	A65-4-1+2				Neutral	>20	>2.5	SAKNFYDWVYKK (ε -biotin)
D114	IGFR A6	1	8.1					
D115	IGFR D5	1	8.1					
D116	IGFR JBA5	9	4.4 cycli	>20 μM	Agonist	8.1	1.8	ADKNFYDWVMAKK (ε -biotin)
D117	IGFR H2C	1	0.70	YES	-20 μM Agonist	6.1	8.6	DYKDLQCSWGVRCWLAGLCPKK (ε -biotin)
						5.1	8.5	FHENFTYFWRQVSKK (ε -biotin)
D118	20E2	2	0.25	YES	-20 μM Agonist	1.3	5.2	DYKDFYDAIDOLVRSARAGGTDRKK (ε -biotin)
D119	20C11	2	0.25	YES	-20 μM Agonist	1.3	2.9	KDRAYNGLRLVGAIVGAWDKK (ε -biotin)
D120	E8	10	0.57					KVRGFQCCIWPCYEWLRNAKK (ε -biotin)
D121	F2	10	1.1					KSMFYAGSDRWPYCIVLAOWLKK (ε -biotin)
D122	20A4 (A7)	6	1.2					KEIEAEWGRVRCIVYGRCVGGKK (ε -biotin)
D123	D8	6	0.55					KWLDQEWAHQCEVYGRCCPSKK (ε -biotin)
			1.3					
D124	F8	4	0.04*					DYKDFERSAGFRGNFYDWVQAQVNKK (ε -biotin)
D125	IGFR E4	1	2.6					
D126	IGFR D2C	1	1.4					LGEMFYDWVFMQVRKK

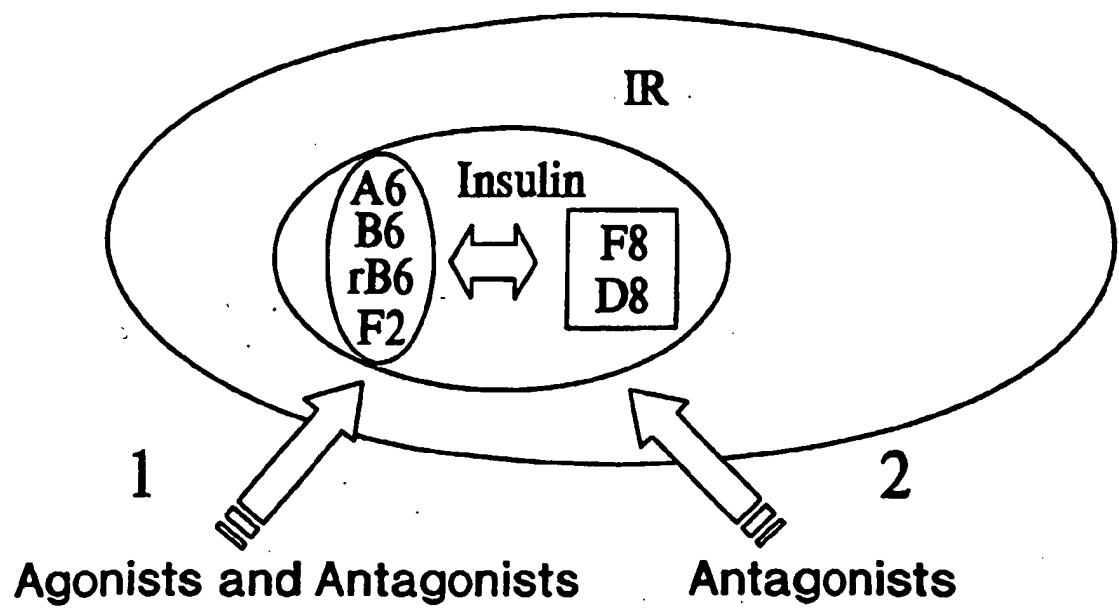
FIG. 5A

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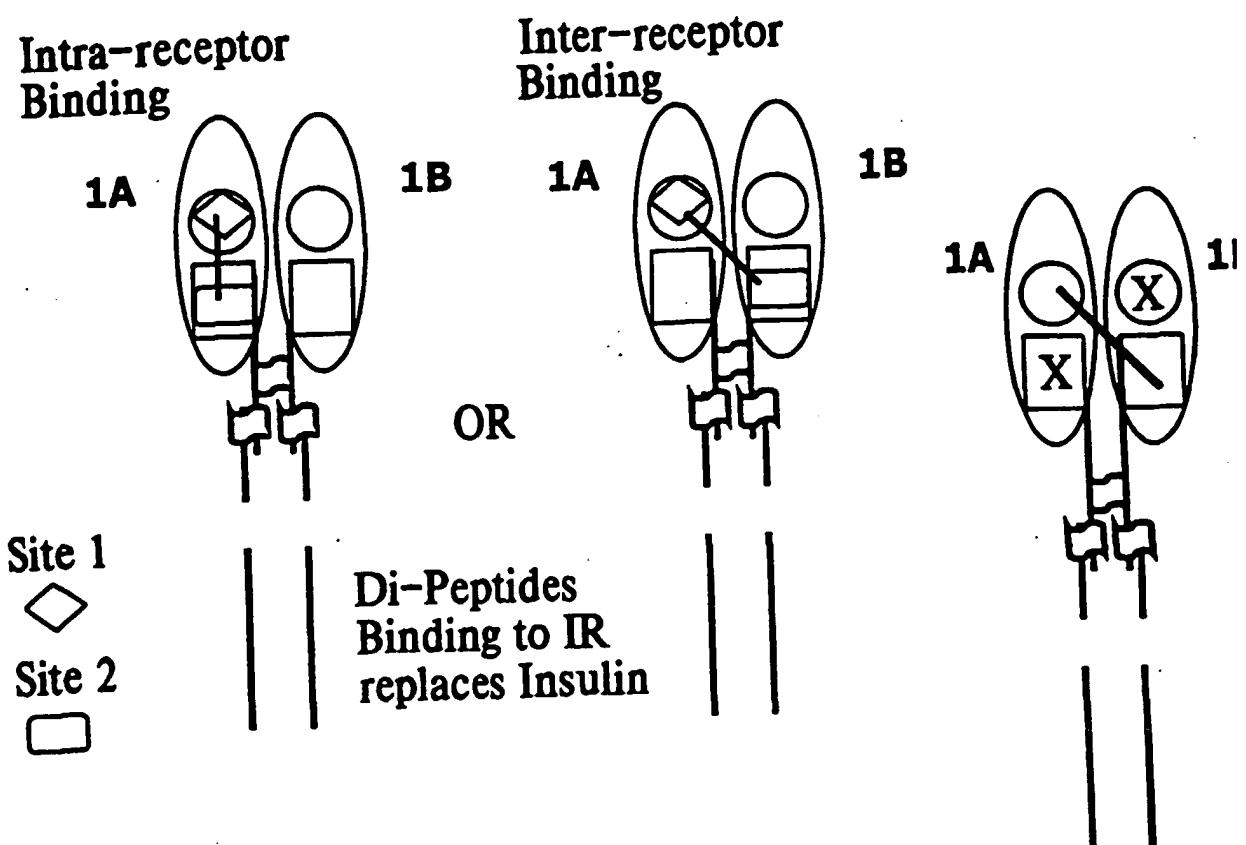
Clonal Name	D or S	Motif	Sequence	TR-Kd	IR-TC50 Biocytin	IR-TC50 FP- Biocytin	P04	Fat Cell Assay
20-E2	D118	B6	DYKDFYDAIDQLVLRGSARAGGTROK	K-biotin	250 nM	2.8 nM	+	++
C1	D112	A6	DYKDFCWARPGCGAANFYDWPGQAS	RK-biotin	490 nM	-	0	-
D8	D123	C-C	C-LOOP	KWLDOEWAAYVCEVYGRGCPSSK	350 nM	0	-	-
F8	D120	GROUP 6	KRGFOGGTYWPGYEWLRNA	370 nM	-	-	-	-
F8	D124	C-C	C-LOOP	KHICMEEELFWCASLFGCYSGKK	40 nM	-	0	++
H2C	D117	A6	FHENFQWAVYDQSVKK	700 nM	>5 μM	5 nM	++	++
KC19			RILYEFWFQGQEAQGRGGLS					
KC-C2		C-C-C	GLEQCCPFWVGLFEVQRCGCPSS					
KCG7		B6	FYCGGLEELSWGAALFGYCSGC		>1 μM			
NG-C2		B6	CNGDGWYFYLSSLLVGRDWH					
NG-G3		A6	CHSSOSCPESTYDWFAGQVSDPWVWCW		2-4 μM	4.2 nM	++	++
NG-C8		B6	VEGRGLFYDILLRLLARRQNG		>5 μM	-		
NG-G9		B6	RAMSFYDALWVGLCPKK-Biotin					
RP-1		A6	GSRPVFTHEQFYIWFVDOLQCL					
RP-2		A6	RSEASFLIVEFYFWFEQQLRSF		1 μM	+	+	
RP-3		A6	GRFTGWFQDAIDQMLPWGFD		1 μM	+	+	
RP-4		B6	PPWGARFYDATEIQLVFDNL		>10 μM	-		
RP-5		B6	AGVAVAGTFTYSILIDWWDQCKKK-Biotin		5 μM	+	+	
RP-6		B6+	TFYSCLASILTCPOPNRGPWERCRKK-Biotin		6 μM	-		
		C-C			>5 μM	++	++	
RP-7		A6	AAVHTOFYDWTADQYKK					
RP-8		B6	QSFYDYEELIGCFWKK					
RP-8'		B6	QSFYDYEELIGCFWKK		>5 μM	+	+	
RP-9		A6	GSLOESFYDWFTRQGKK					
RP-10		B6	GSPYEALQRLVGEGQCKK		>10 μM	+	+	
RP-11		A6	QAPSNTFYDWFVREWKK		>10 μM	+	+	
RP-12		B6	DPTYQGWLWLTIESGKK					
RP-13		A6	ASGFPEFYDWFGRQLSLKK		>10 μM			
RP-14		A6	SACQTDCHENFTDWFAROKK		>10 μM			
RP-15		A6	SOAGSAYAWTDDVLRTVKK					
RP-16		B6	YMDARDDPYHKLSELVTKK					
RP-17		B6	OSDAYSGCLWATIGLSGKK					
RP-18		B6	LOPSCGPYDWFWRTHLGSKK					
RP-19		A6	KKQGDTDTWQRLHLGSKK					
RP-20		B6	GSASFSDADRLRMRKK					
RP-24		GROUP 6	WPQGTLFFELAQWGRSTD					
S16		A6	APYDWWAKK		>20 μM	No Binding		
S17		RBB	CDALDRILRYTECERPSL		12 μM			
S17'		RBB	PLAEFLWATPHSCGRSSAH		16 μM		0	0
S175		A6	GRDWVQRNANFYDOWVIAELC		2.90 nM	2-4 μM	0.9 nM	++
S176		A6	GRDPRAGGDNFTDWFVQAH		170 nM			++

5B
FIG.

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**FIG. 6**

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**FIG. 7**

	Target	Found	IR	IGF
20D3*	IGGQQ <u>Q</u> HQDGNNFYDWEEV VFWNCRSQQLDFYEWETEQAA	18	+	++++
20F1	RGGGT <u>F</u> YEWETE <u>S</u> ALRKHGAG	16	+	+++
G3		8	+	++
20H1	RWAGAISAPGLVSNK <u>Q</u> DGLFYSWRE	5	+	+++
20D1*	VL <u>Q</u> ARHGCDSVSDCFYEWFA	4	+	+++
D2	DPERMQSDVGFYEWFRRAVG	3	+	++
B8	WSALLSVMDTGFYAWEDDAV	2	+	++
C4	DIGSDGHGRRWDSSFYRWTEM	2	+	++
A8	IGGSFVEFYGWENDQV	2	+	++
E7	GHSWALVRHVDRFLFYEWFDL	1	+	++
C8	LPAGGAQGFAVRGFYEWETES	1	+	++
H8	RDKPTDQEEQNWSFYEWFRH	1	+	++
E2	SRDQTNTENSAGFYGWTER	1	+	++
	GAFYRWFHEALVGSERVPDV	1	+	++
B12	RIGGGWARSEGFYEWTFVREL	1	+	++
D10-2	RMFYEWTFWSQMAGGPTEGSA	1	+	++
G8	HEAFYDWFSSALVDGGYELMG	1	+	++
H3	FYGWFSRQLSLTPRDWGLP	1	+	++
3G11		1	+	++
F4	GVGTLTMSSDAFYTWEV	1	+	++
E7-2	LGTSAQQGVGHRAFTYQWFQS	1	+	++
40G11	<--ETLESHVVVP <u>Q</u> -- IRDMMHXYWV <u>Q</u> DRDRYINGVR <u>Q</u> WYISDRYNPGSAFYRWFD	3	+	++
40B2	RMGL <u>Q</u> ALAHAHRKSA-----GPIFISSGSVIKGSEGDPFYAWFRL <u>Q</u>	2	+	++
40B12		1	+	++
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FIG. 8

Group 2: Formula 6 Motif

	Target	Found	IR	IGF
20A4*	EIEAEWGRVRCLVYGRCVGG	13	+++	0
D8	<u>WLDQEWA</u> WQCEVYGRG <u>CPS</u>	3	+++	?

Target

	Target	Found	IR	IGF
		13	+++	0
		3	+++	?

Group 3: Formula 2 Motif

	Target	Found	IR	IGF
20E2	DYKDFYDAIDQLVRCGSARAGGTRD	1	+	++++
20C11	DYKDDRAFYNGLRLDVGA ^V YGAWD	1	+	++++
20A12	DYKDRFLFYCGI <u>Q</u> ALGANLGYSGCV	1	+	++++
C6	DYKDFYSALIWGLCGVTGCC	1	+	+++
A6	RQOSDAFYSGIWLALIGLSDG	1	+	+++
40H4	RYFPFGGFYGNILDVLRWLRPYVASPRWGCHWRPGCSSLGKQPT	1	+	0

Target

	Target	Found	IR	IGF
		13	+++	0
		3	+++	?

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Group 5: Miscellaneous Motif 10

	Target	Found	IR	IGF
D9-2	PFGFGGRWWGIPRMWYRNS	1	++	+
H4	WWGGGRNRNFWLTERWGLGGER	1	+	+

Target

	Target	Found	IR	IGF
		13	+++	0
		3	+++	?

FIG. 9A

Group 4 and 6: Miscellaneous Motif 10

	Target	Found	IR	IGF
D10	LGPLLRRWGSEVCGWPDLC	3	++	0
A2	GRVALWGPVWPRWMFMSRPV	1	++	+
F2	SMFVAGSDRWPGYGVLDWL	1	++	++
E8	VRGFQQGTVWPGYEWLRNAA	1	++	?
A4	WPGYLFEEALQDWRGSTED	1	0	++

Group 7: Formula 4 Motif

	Target	Found	IR	IGF
B6	ACSSFFVKKGPEGFLQCLGSI	1	0	++
F8	HLCVLELFWGASLFGYCSG	4	+++	+
40D6	PERGRGLRTAMQLMRRPRDWPHFPHSLFWGAPPPLSG	1	0	0

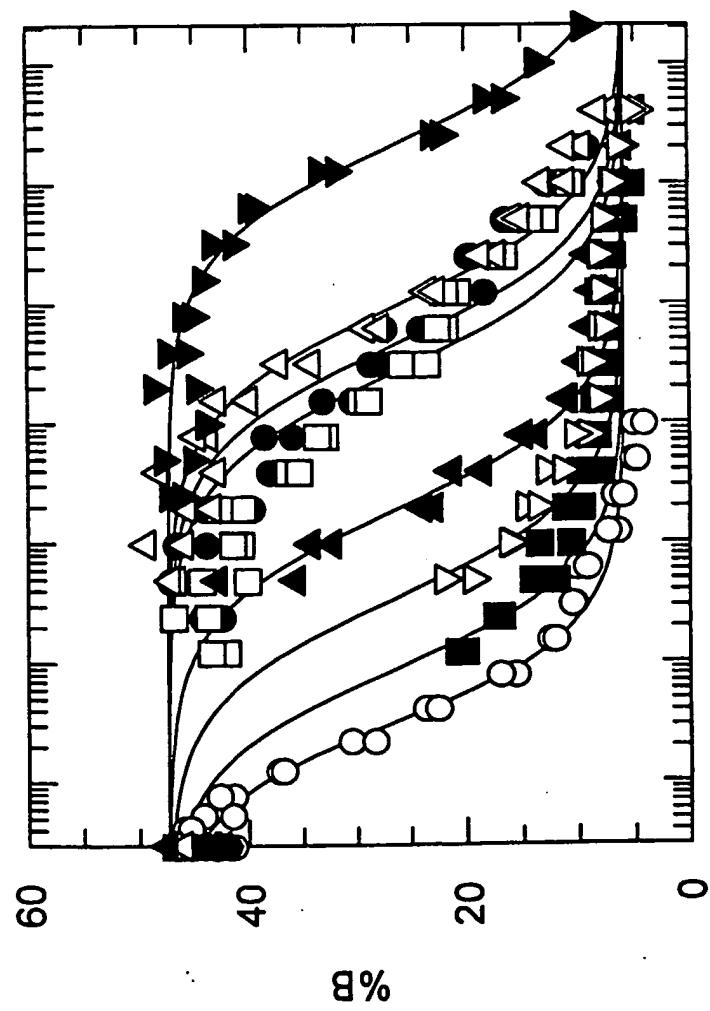
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	Target	Found	IR	IGF
		1	0	++

FIG. 9B

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FIG. 10A
Displacement of ^{125}I -HI from HIR-11



1e-0111e-0101e-0091e-0081e-0071e-0061e-005

[M]

FIG. 10B

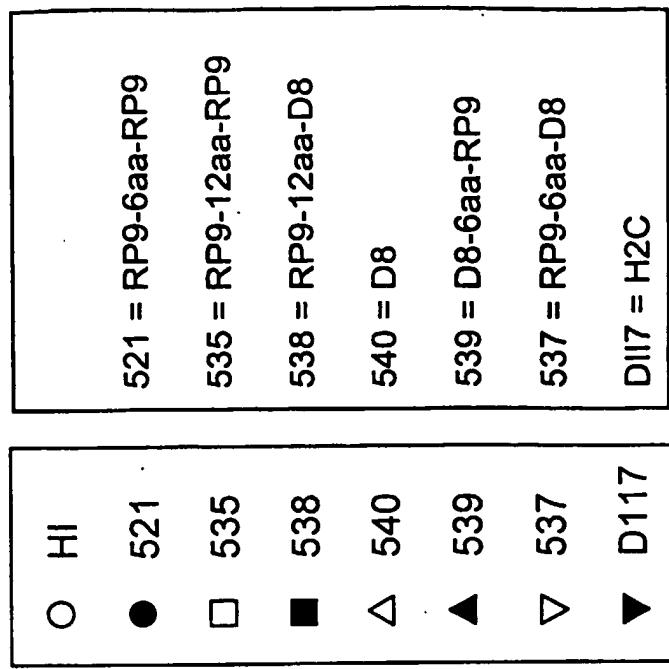


FIG. 10C

FIG. 11A

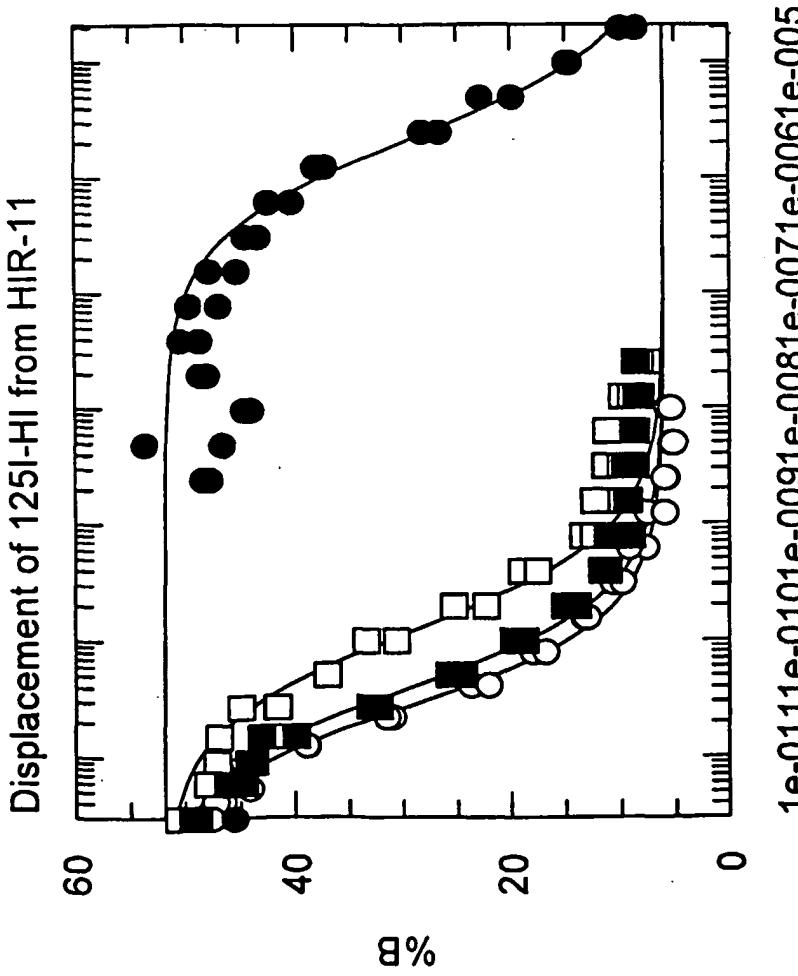


FIG. 11B
FIG. 11C

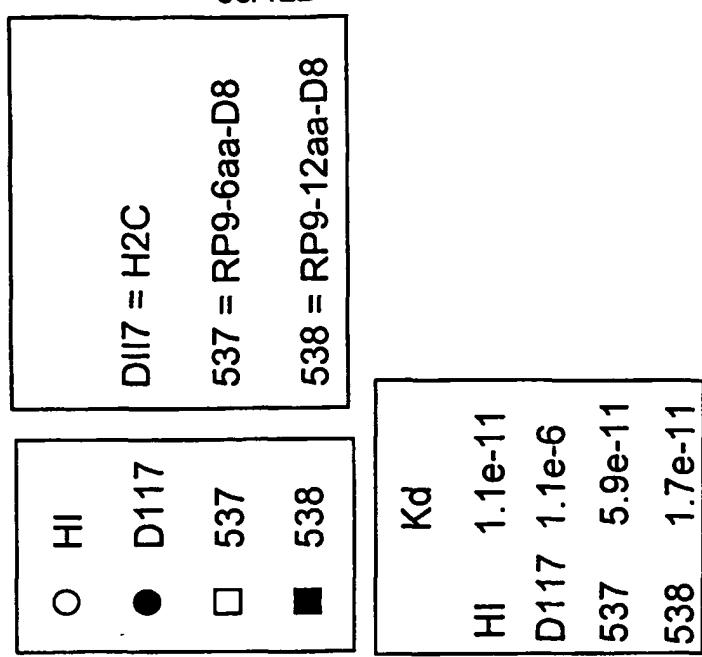
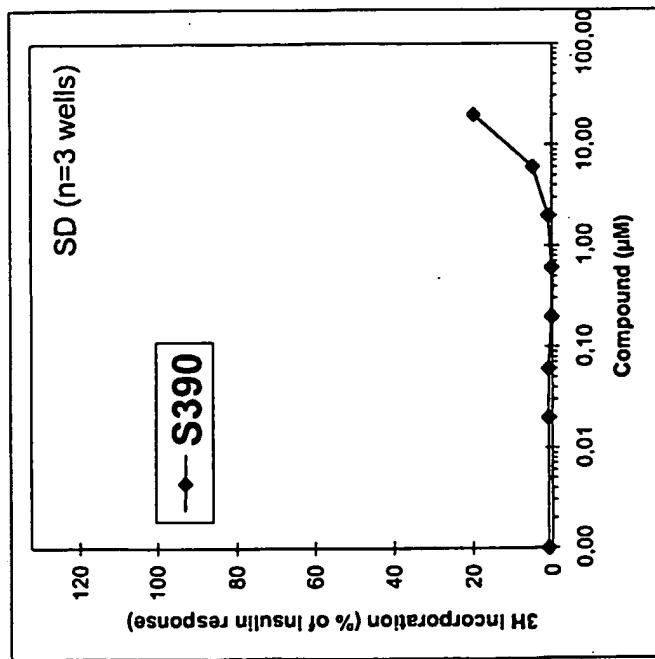
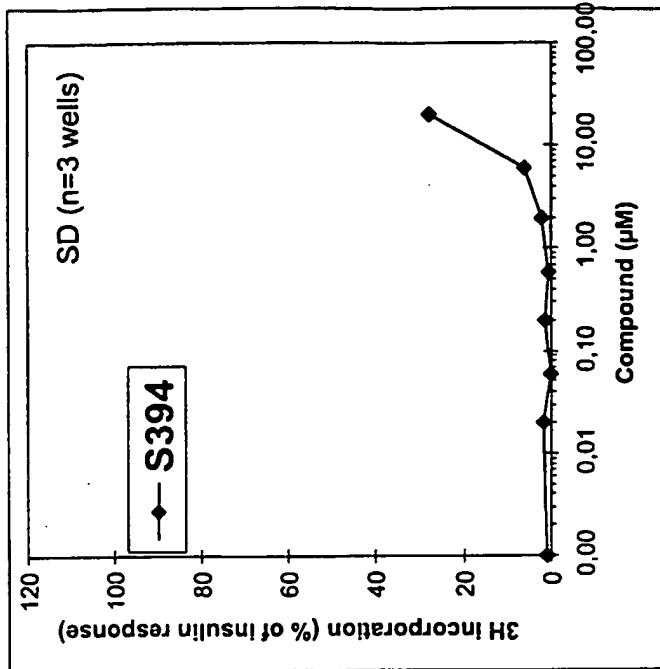


FIG. 11D

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FIG. 12A**FIG. 12B**

S390 = ESFYDWFERQLG
S394 = GSLDESFYDWFERQ

FIG. 12C

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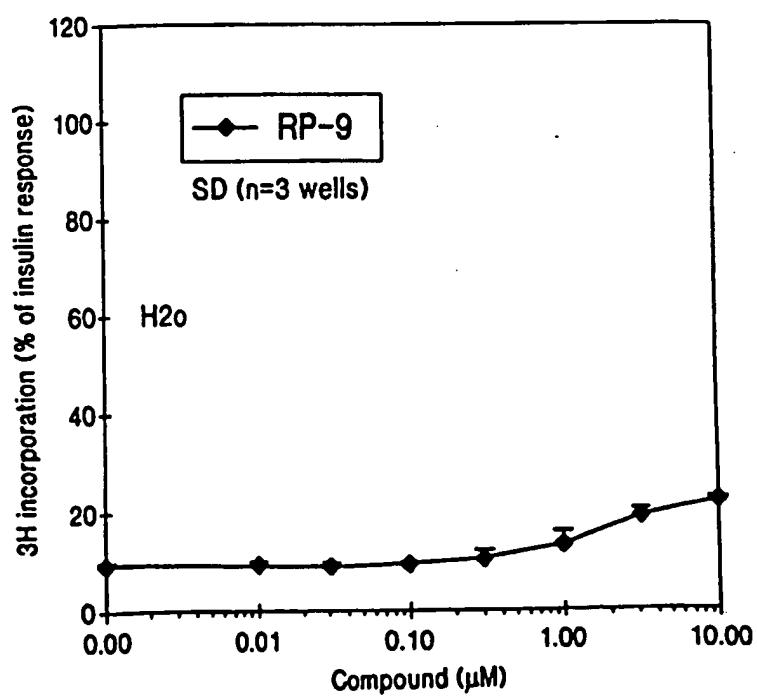
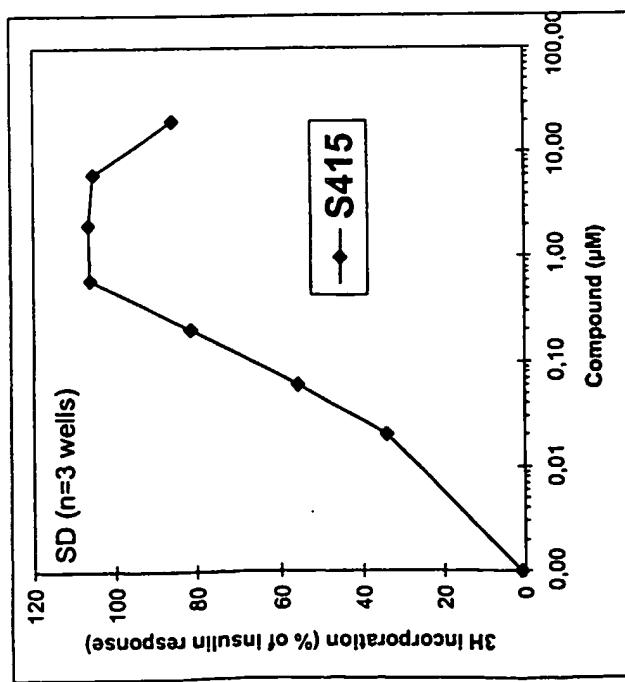
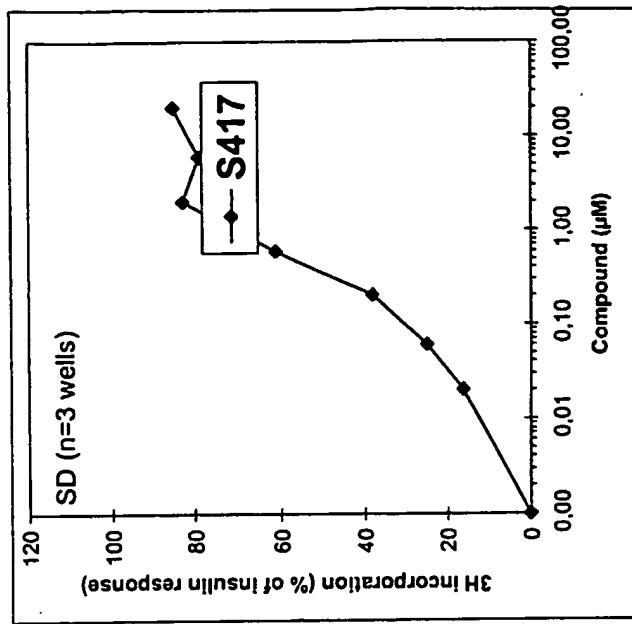


FIG. 12D

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FIG. 13A

S415 $(\text{ESFYDWFERQLGK})_2$ -23
 S417 23-(ESFYDWFERQLG) $_2$

FIG. 13C**FIG. 13B**

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FIG. 14A

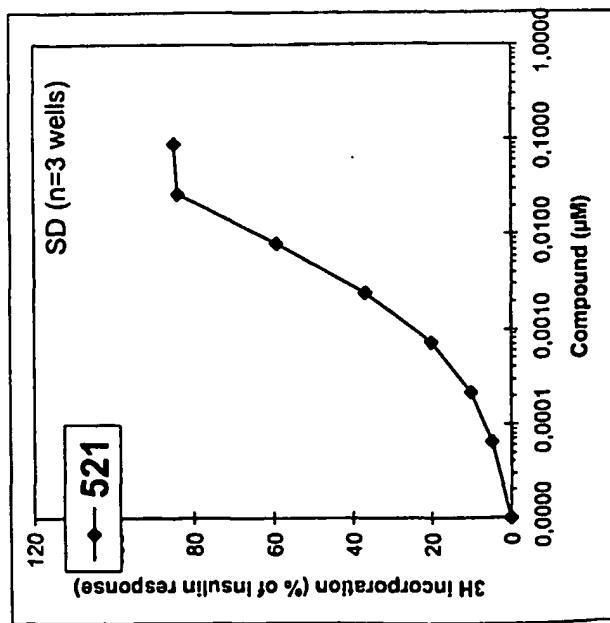
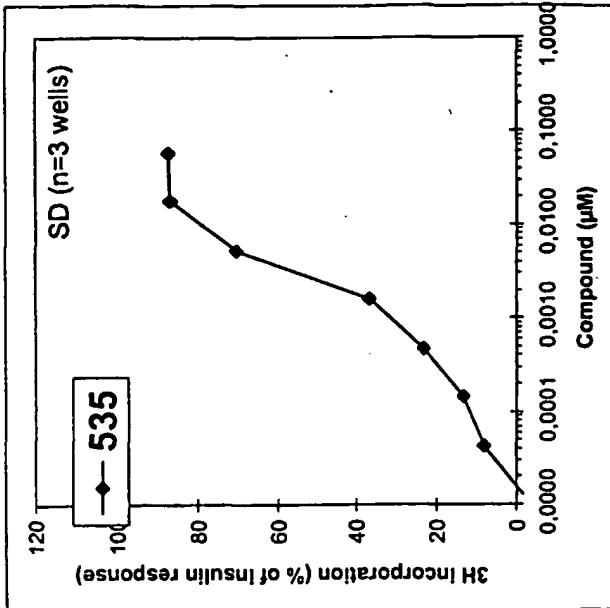


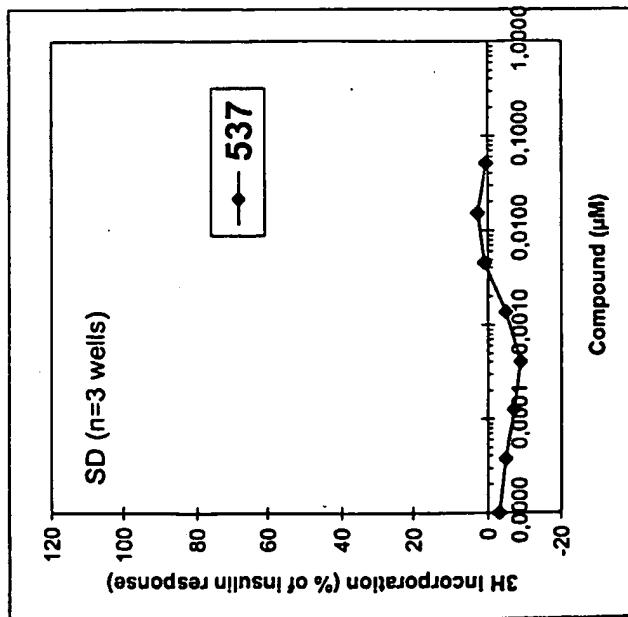
FIG. 14B



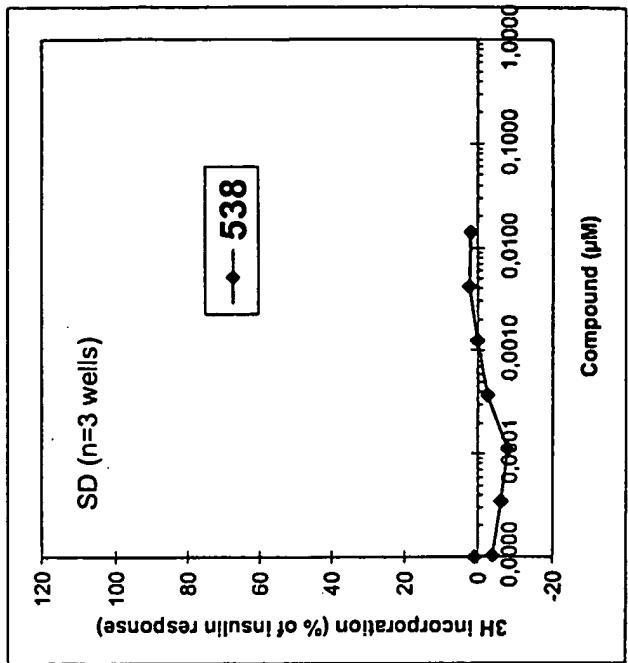
521 = RP9-6aa-RP9
535 = RP9-12aa-RP9

FIG. 14C

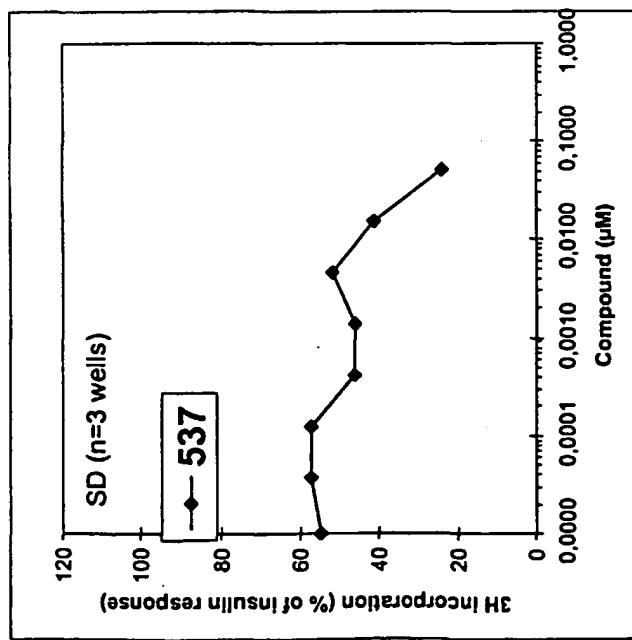
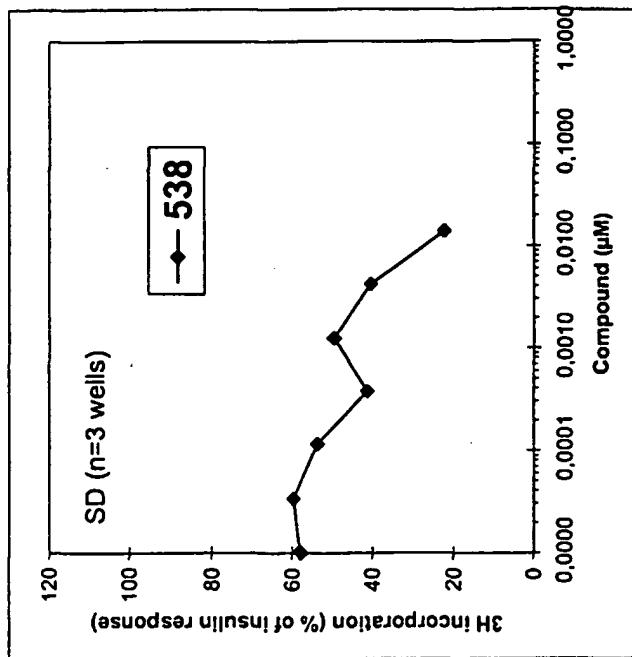
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FIG. 15A

537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 15B**FIG. 15C**

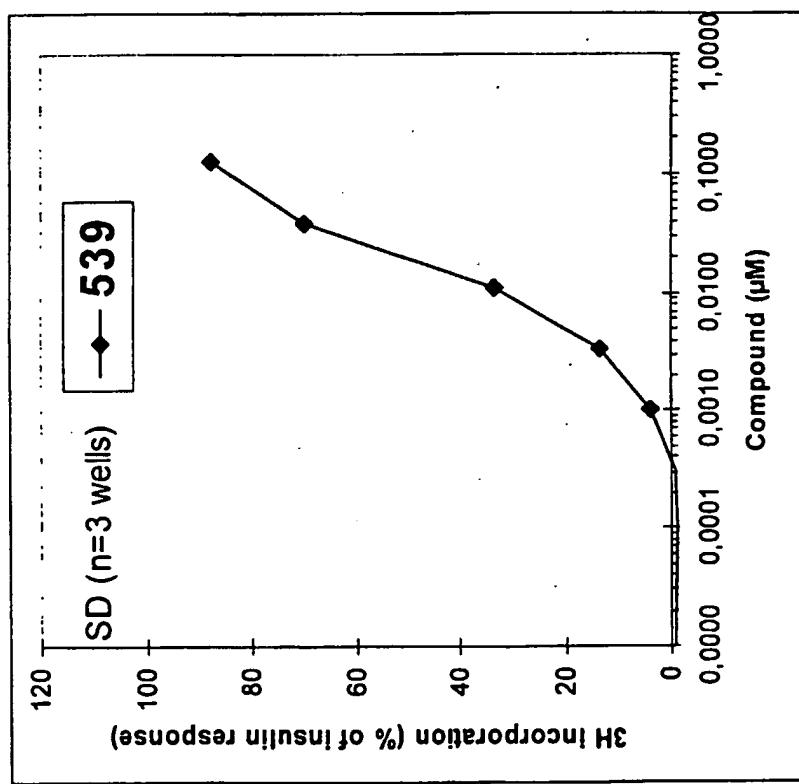
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FIG. 16A**FIG. 16B**

537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 16C

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FIG. 17A**FIG. 17B**

539 = D8-6aa-RP9

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FIG. 18D

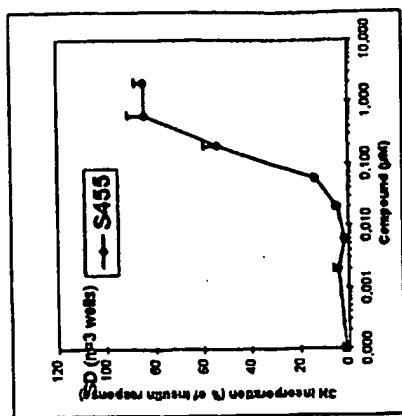


FIG. 18C

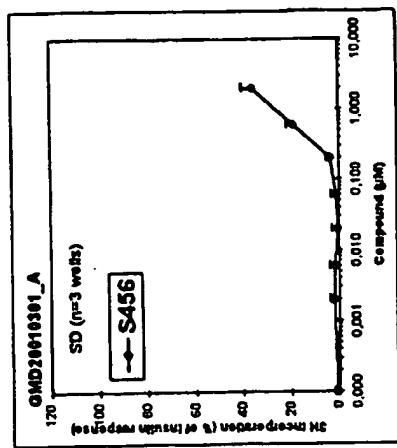


FIG. 18B

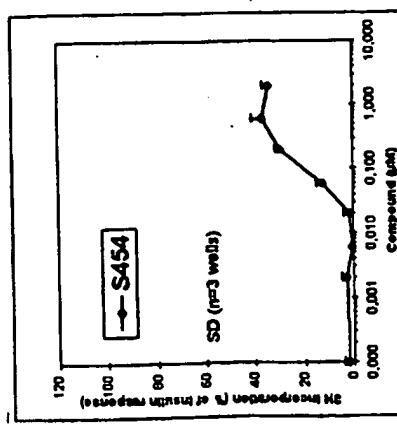
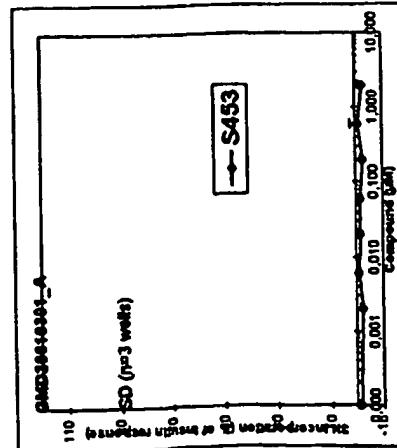
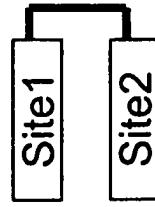


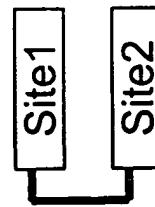
FIG. 18A



N-C



C-C



N-N



C-N

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FIG. 19A

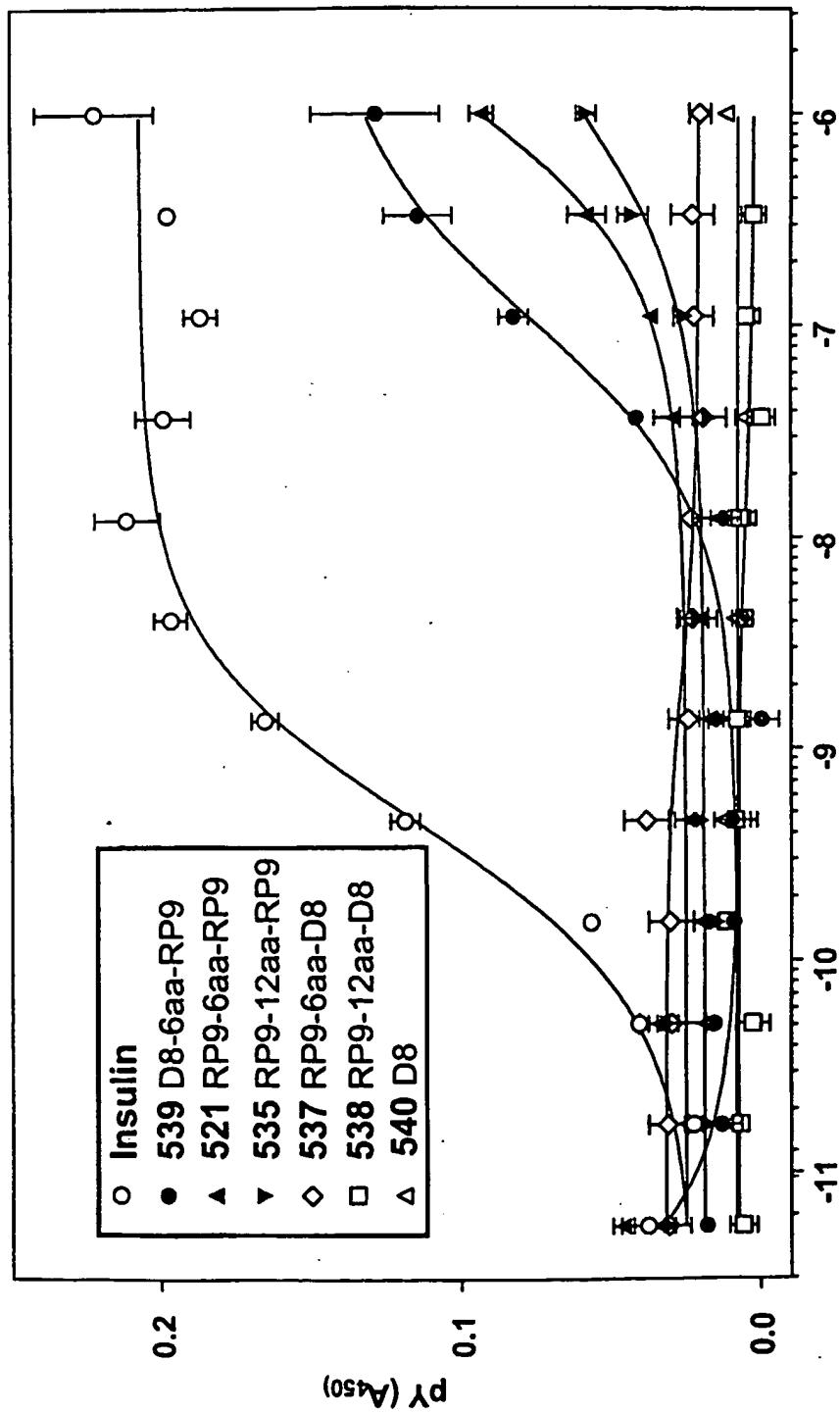
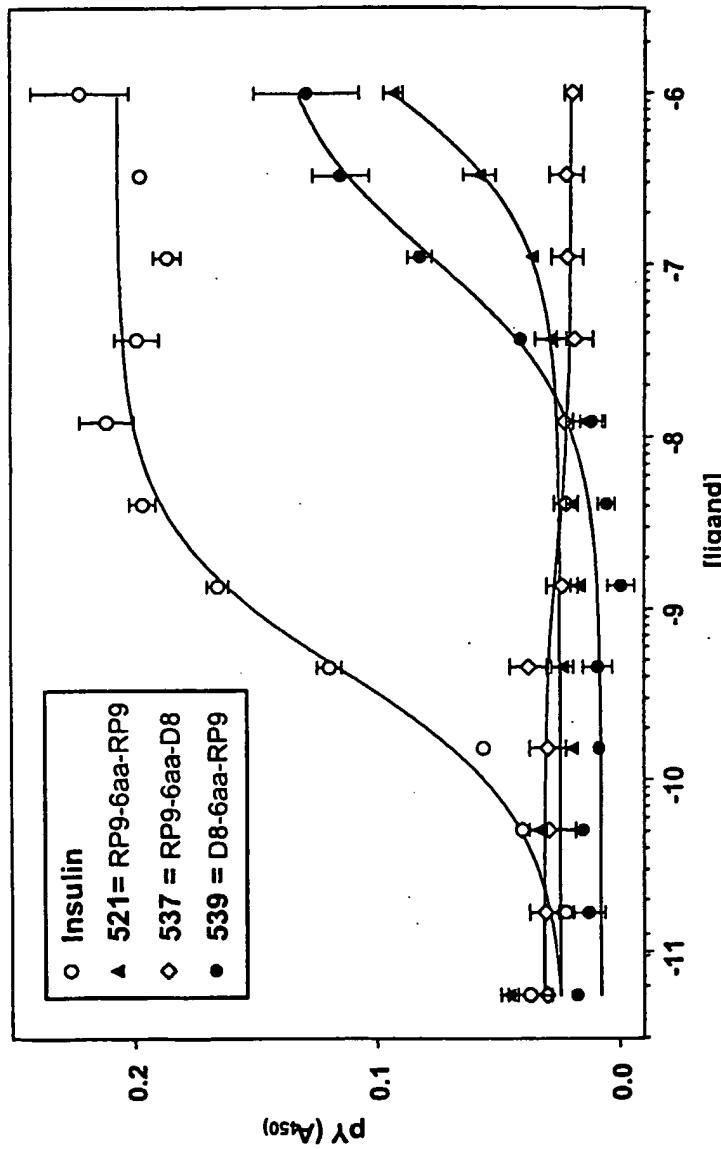


FIG. 19B

Ligand	EC ₅₀	IC ₅₀
Insulin	4.4680e-010	521
539	535	9.6490e-007
521	539	1.1000e-007
535	521	1.4420e-006
537	538	1.4420e-006
538	537	1.4420e-006
540	540	1.4420e-006

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FIG. 20A



	Insulin	521	539
EC50	4.4690e-010	1.4420e-006	1.1000e-007

FIG. 20B

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FIG. 21A

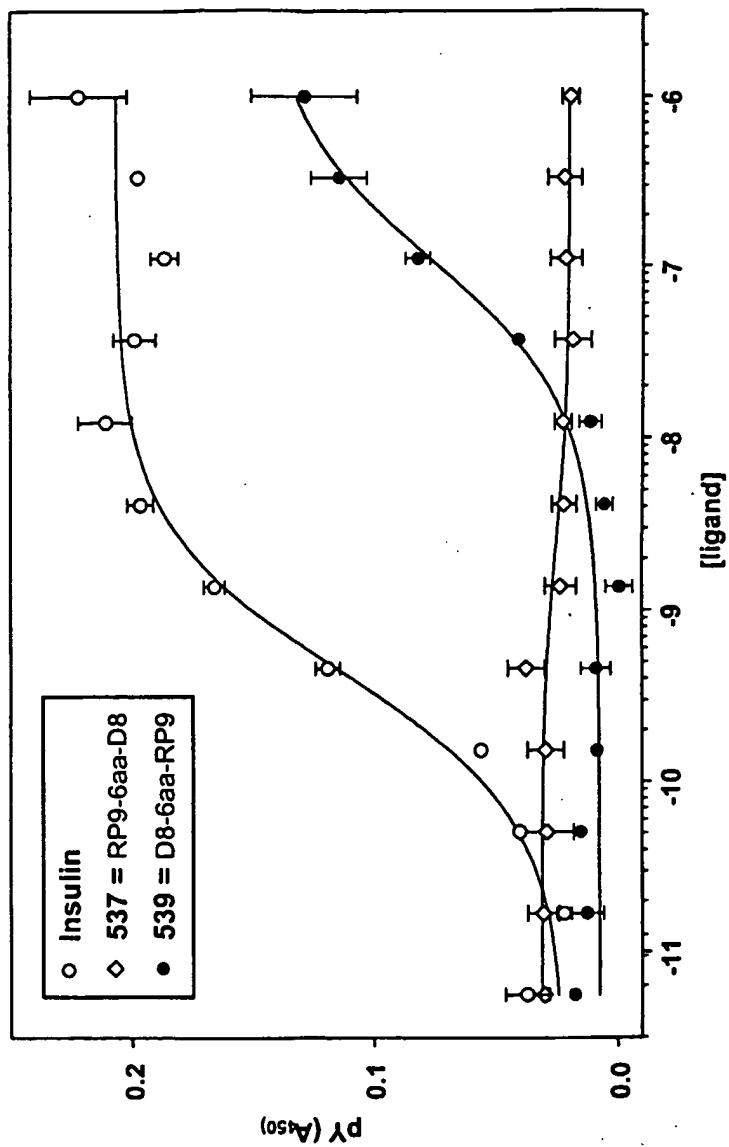


FIG. 21B

EC50	Insulin	539
EC50	4.4680e-010	1.1000e-007

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FIG. 22A

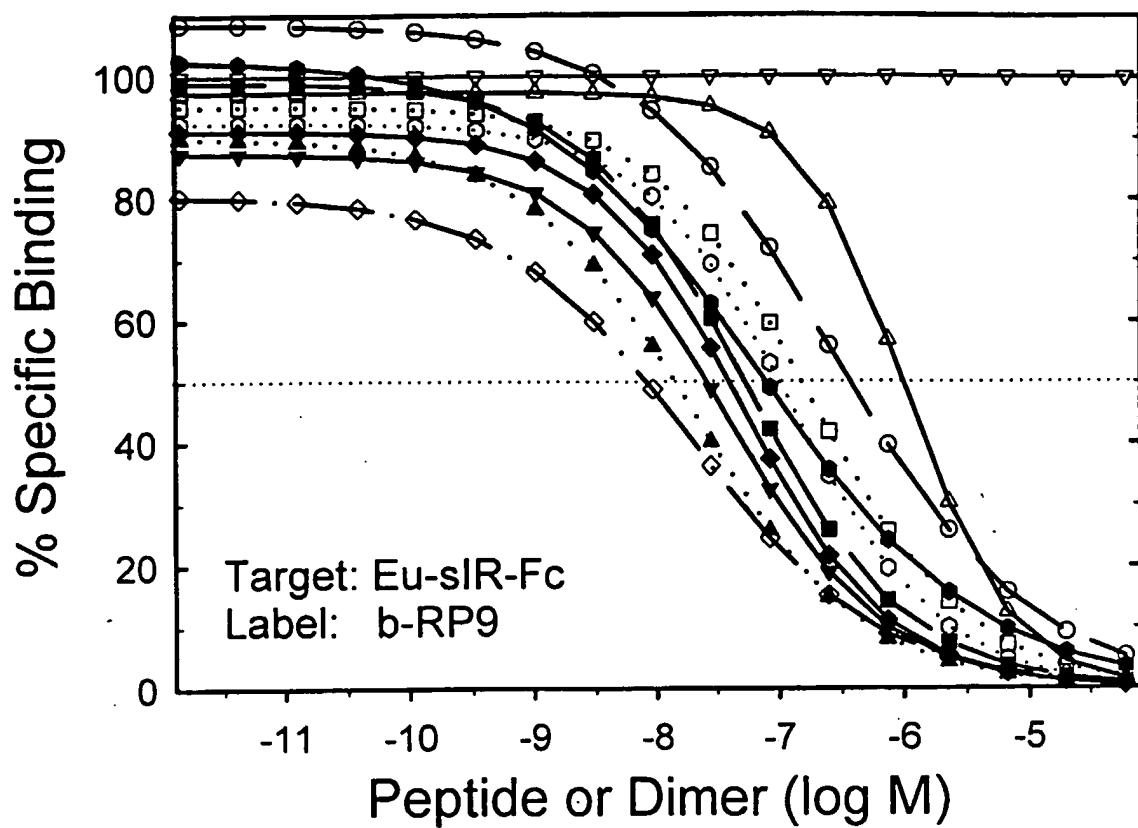
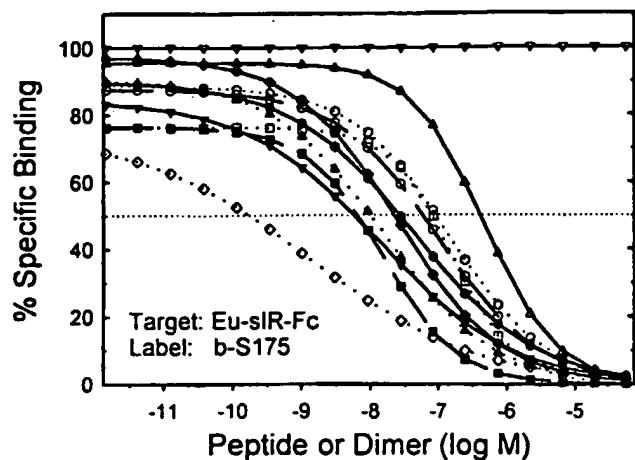
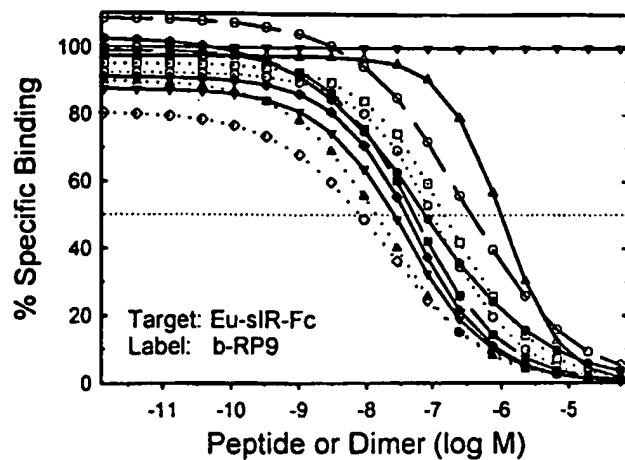


FIG. 22B

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FIG. 23A**FIG. 23B**

Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFWQRVSKK
□	S291	(Lig-GGG-H2C) ₂ -9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)- <u>14</u> -(RP9-Lig)
◊	S337	(RP9-Lig) ₂ -23
○	S391	truncated-(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLL)RP9(-KK)
○	S414	(truncated(-GSLL)RP9(-KK)) ₂ -14
◆	S175	GRVDWLQRNANFYDWFWVAELG
▲	S380	(EE-short-S175-Lig) ₂ -9
▽	Linker 23	

FIG. 23C

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FIG. 24A

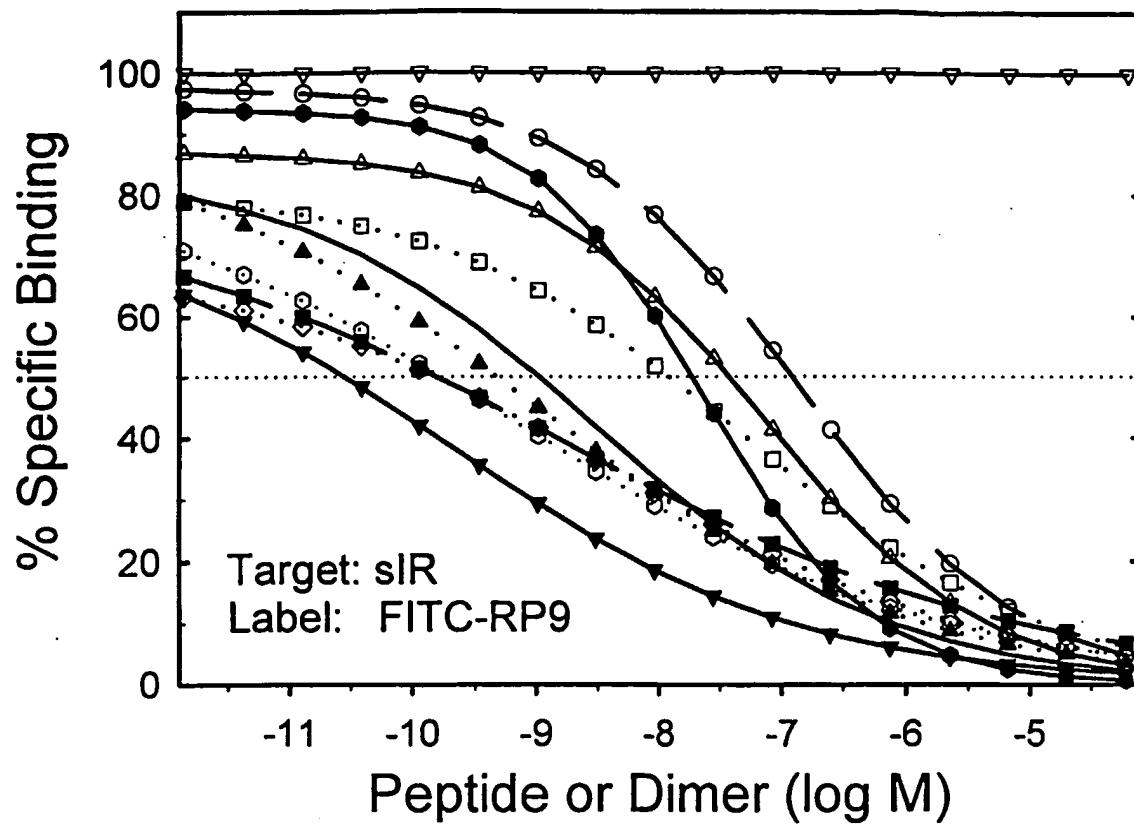
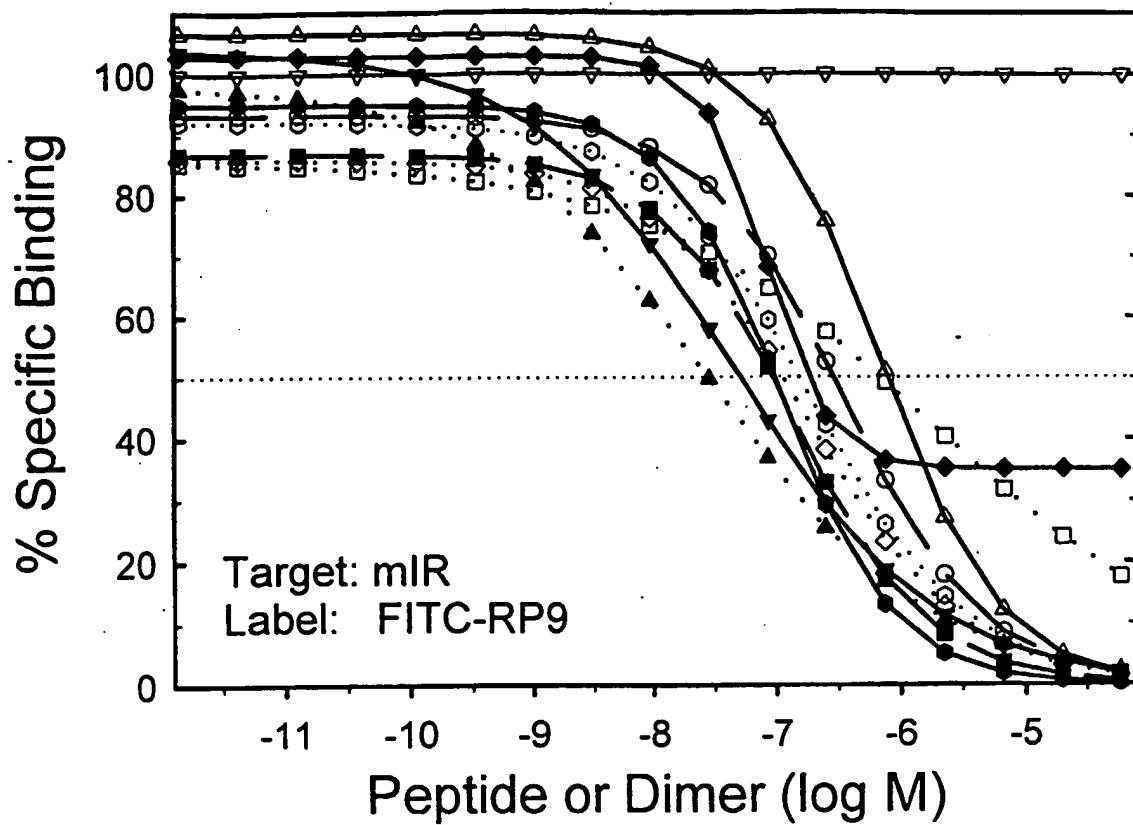


FIG. 24B

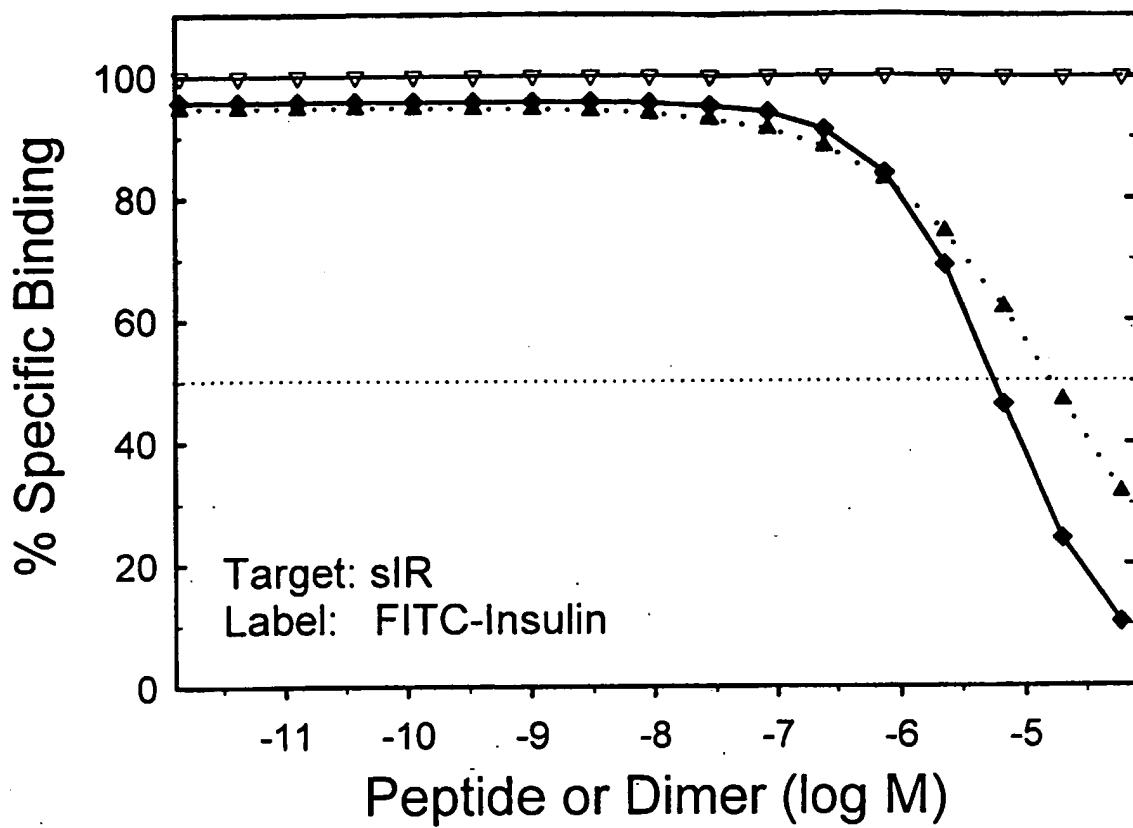
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FIG. 25A

Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDW ¹ FVQRVS ⁹ KK
□	S291	(Lig-GGG-H2C) ₂ -9
▼	RP9	GSLDESFYDW ¹ FERQLG ⁹ KK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◊	S337	(RP9-Lig) ₂ -23
○	S391	truncated-(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
○	S414	(truncated(-GSLD)RP9(-KK)) ₂ -14
◆	S175	GRVDWLQRNANFYDW ¹ FVAELG
▲	S380	(EE-short-S175-Lig) ₂ -9
▽	Linker 23	

FIG. 25B

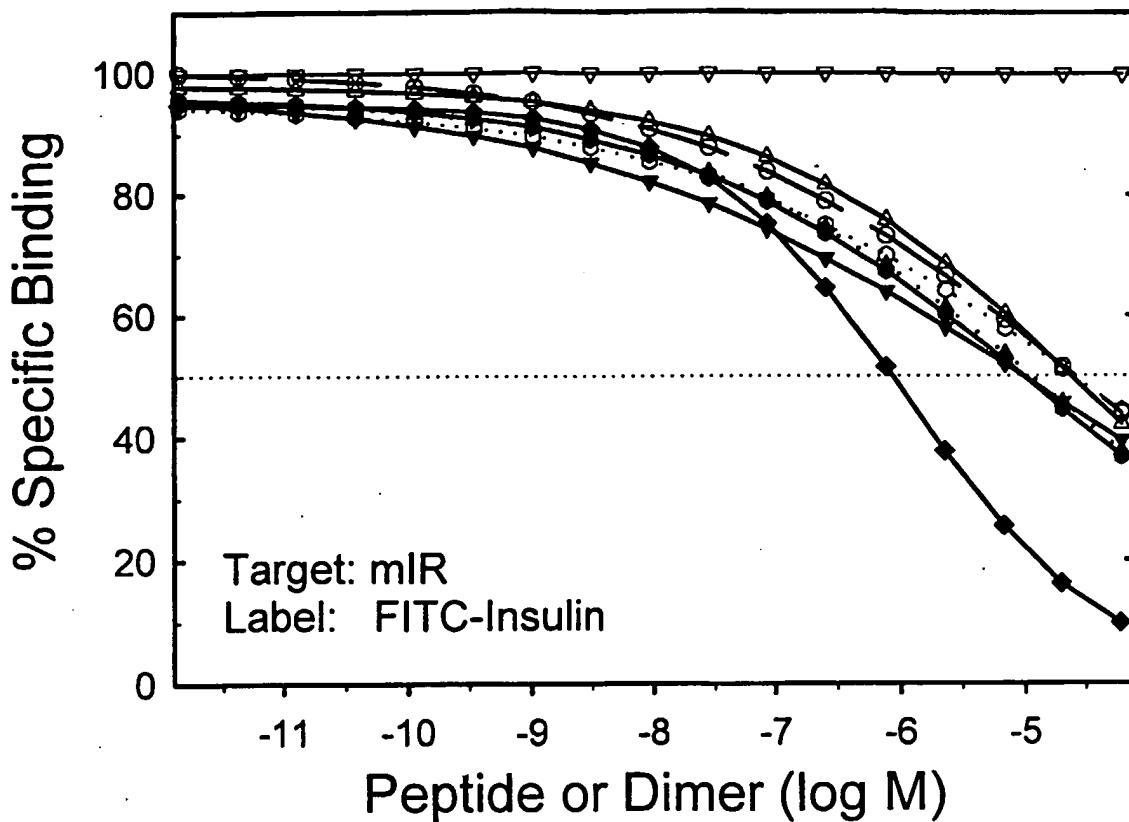
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FIG. 26A**Peptide or Dimer (log M)**

Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFWQRVSKK
□	S291	(Lig-GGG-H2C) ₂ -9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◊	S337	(RP9-Lig) ₂ -23
○	S391	truncated-(-GSLDE)RP9(-KK)
●	S390	truncated-(-GSLD)RP9(-KK)
○	S414	(truncated-(-GSLD)RP9(-KK)) ₂ -14
◆	S175	GRVDWLQRNANFYDWFWVAELG
▲	S380	(EE-short-S175-Lig) ₂ -9
▽	Linker 23	

FIG. 26B

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FIG. 27A**FIG. 27B**

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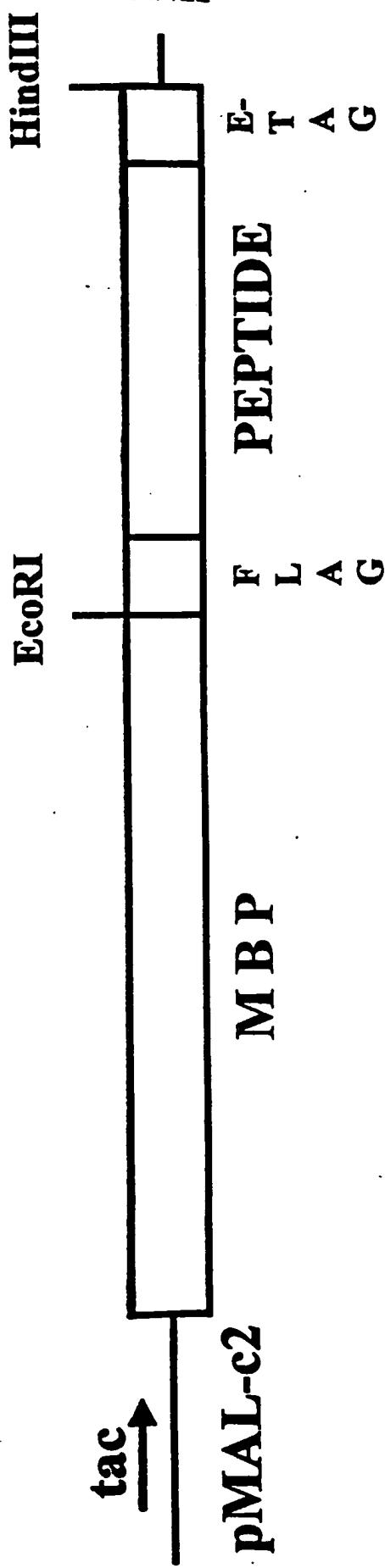
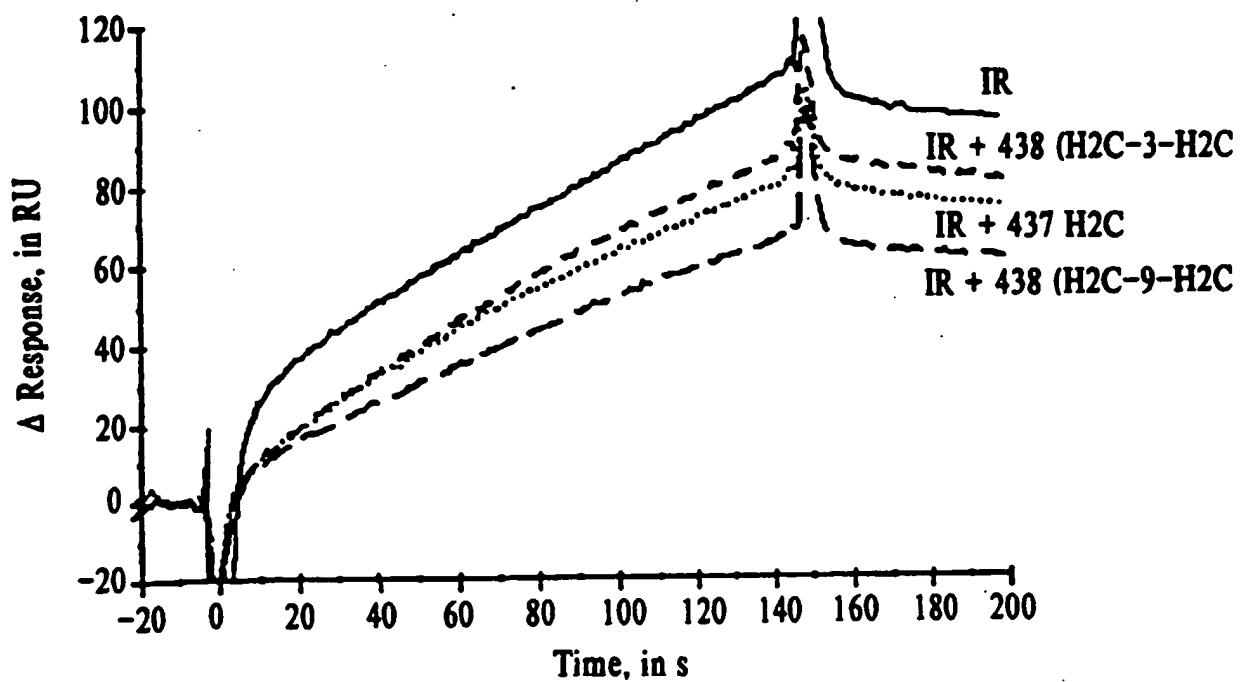


FIG. 28

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MBP-H2C homodimers**FIG. 29**

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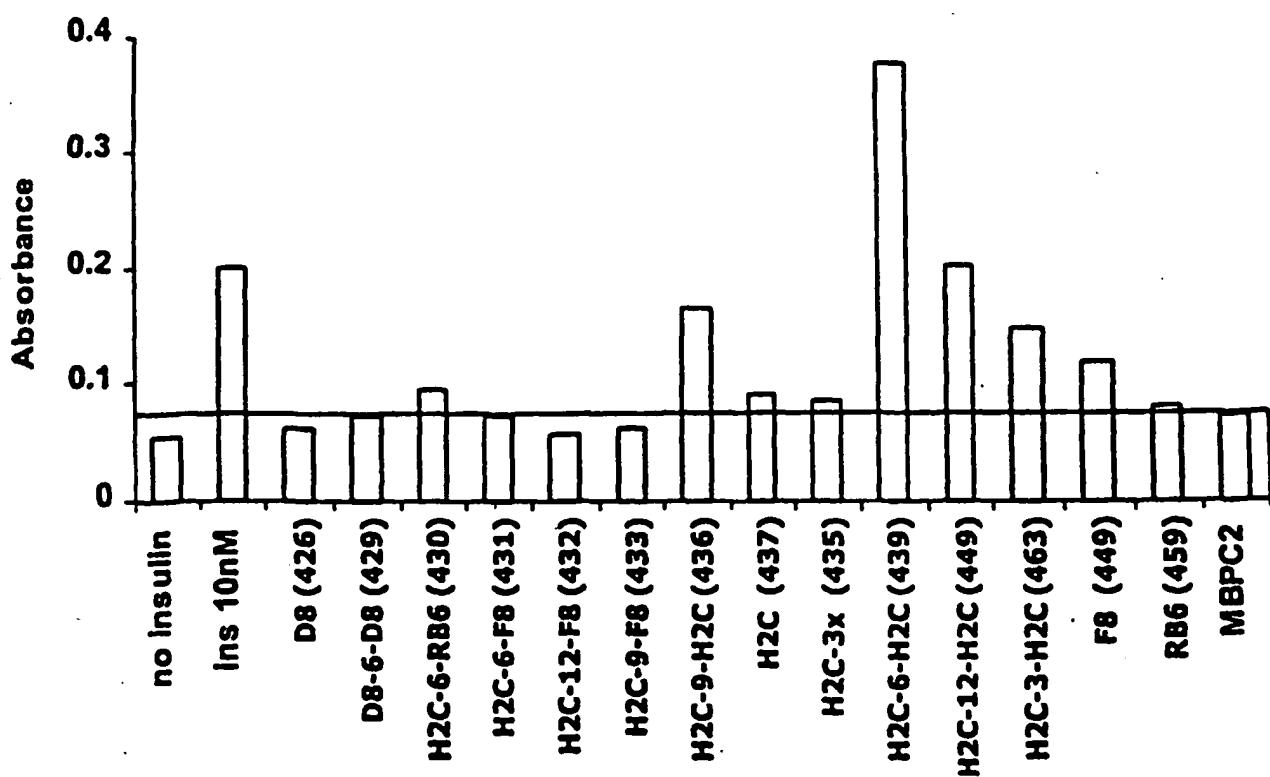
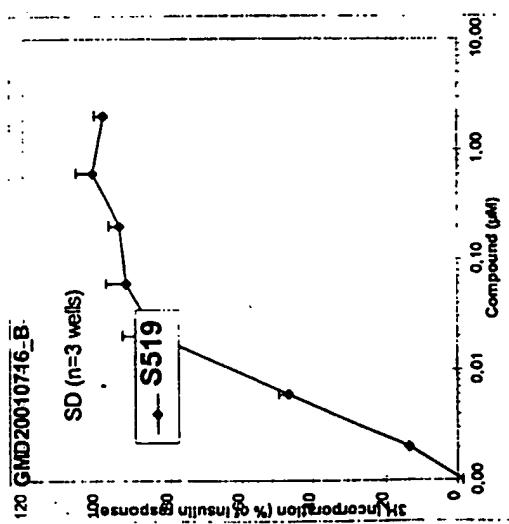
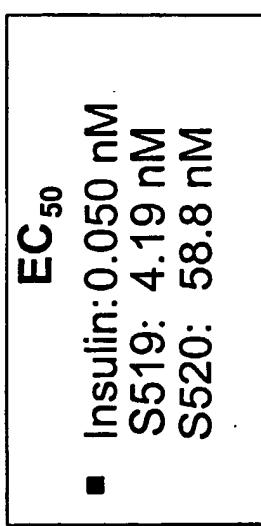
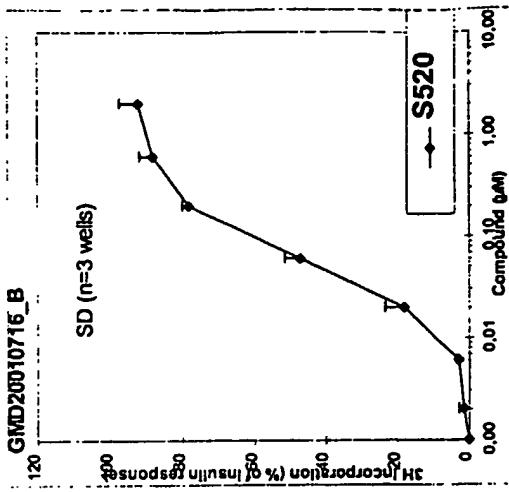
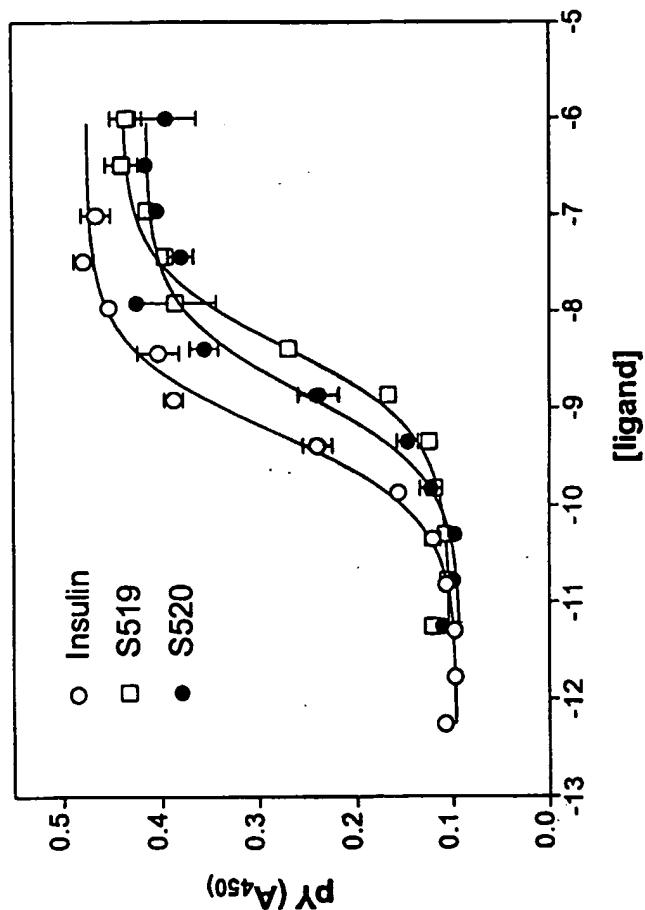


FIG. 30

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FIG. 31A**FIG. 31B****FIG. 31C**

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FIG. 32A

Equation 1	Insulin	S519	S520
Best-fit values			
BOTTOM	0.09614	0.1038	0.09202
TOP	0.4740	0.4388	0.4145
LOGEC50	-9.237	-8.380	-8.852
EC50	5.8000e-010	4.1660e-009	1.4060e-009

FIG. 32B

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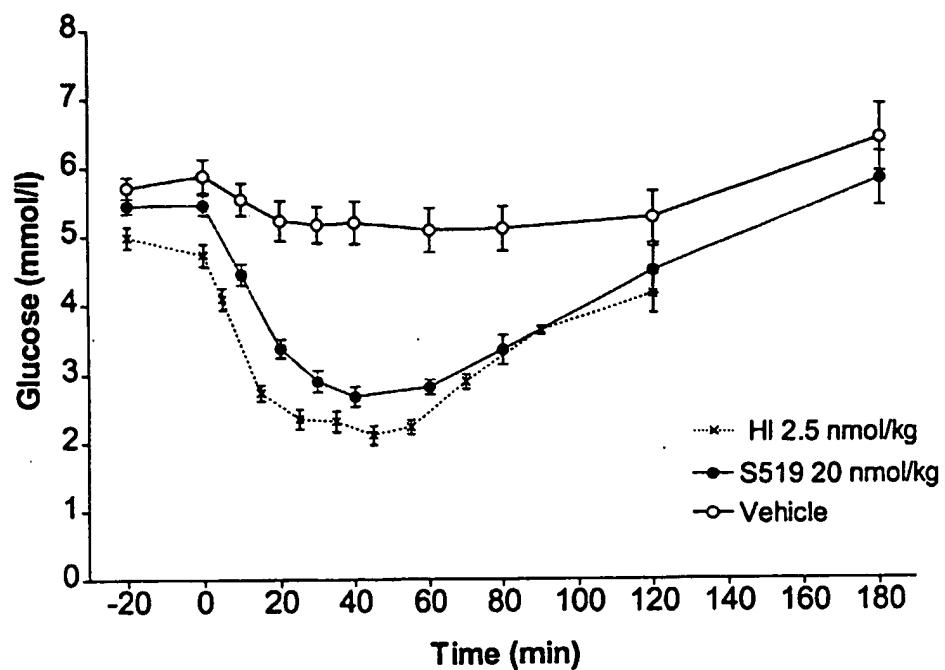
FIG. 33

FIG. 34A

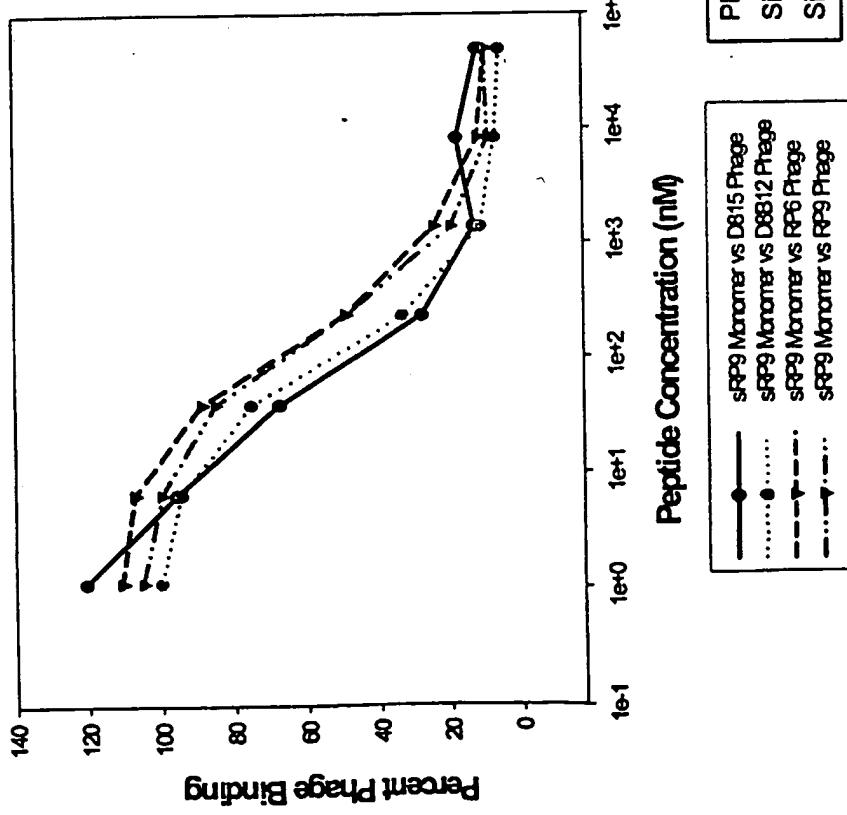


FIG. 34B

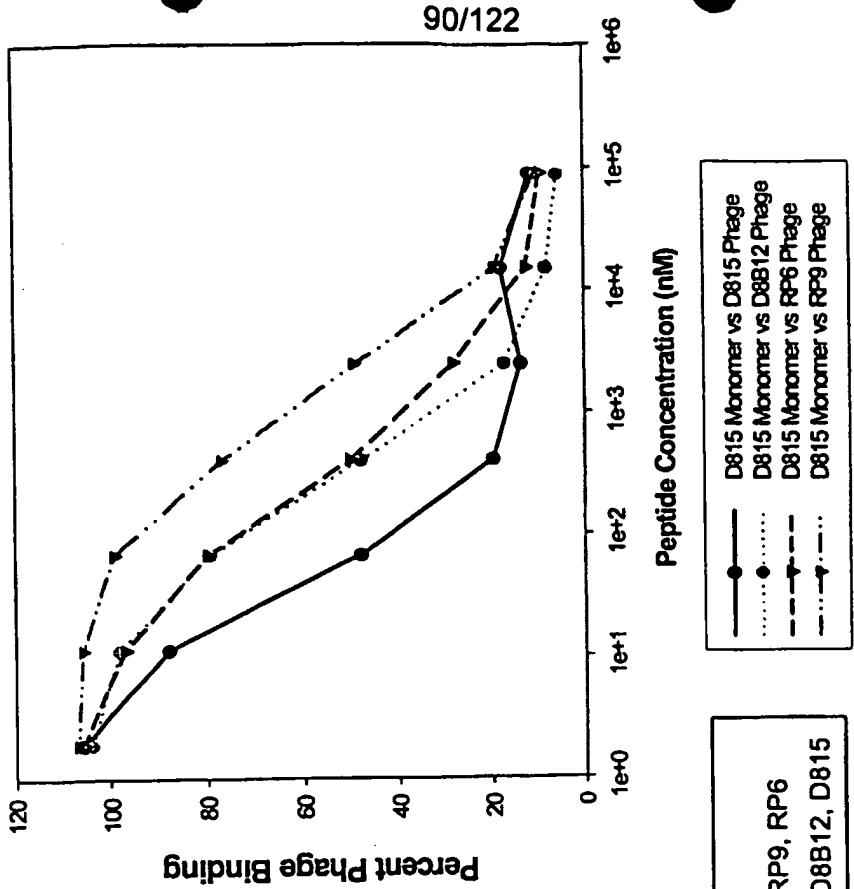


FIG. 34C

FIG. 34D

FIG. 34E

FIG. 35A

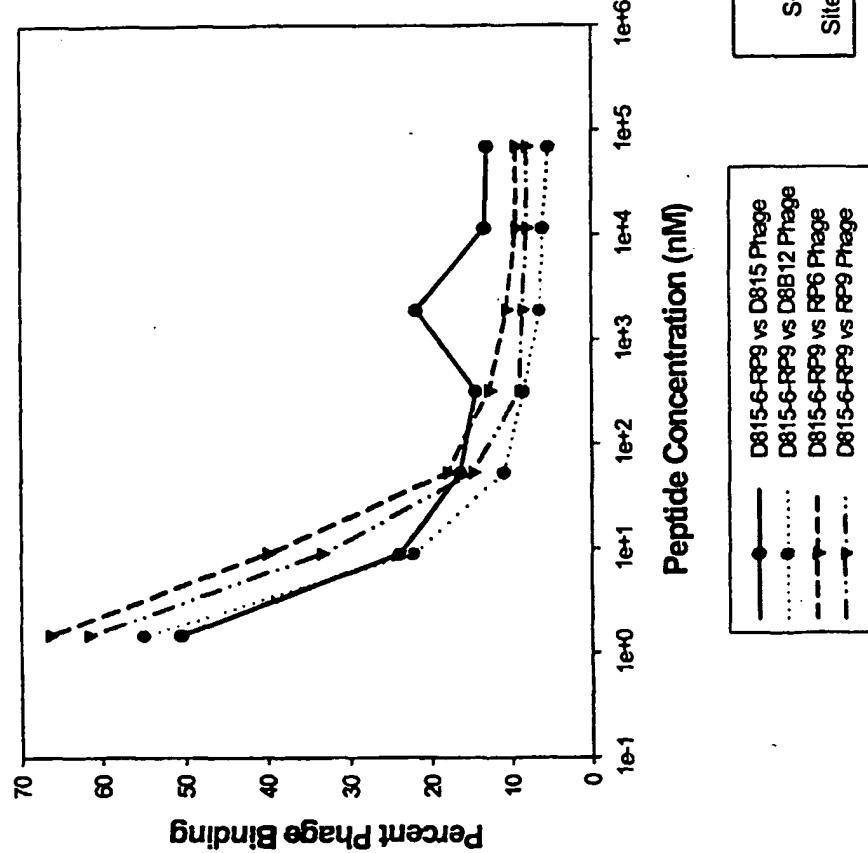


FIG. 35C

FIG. 35B

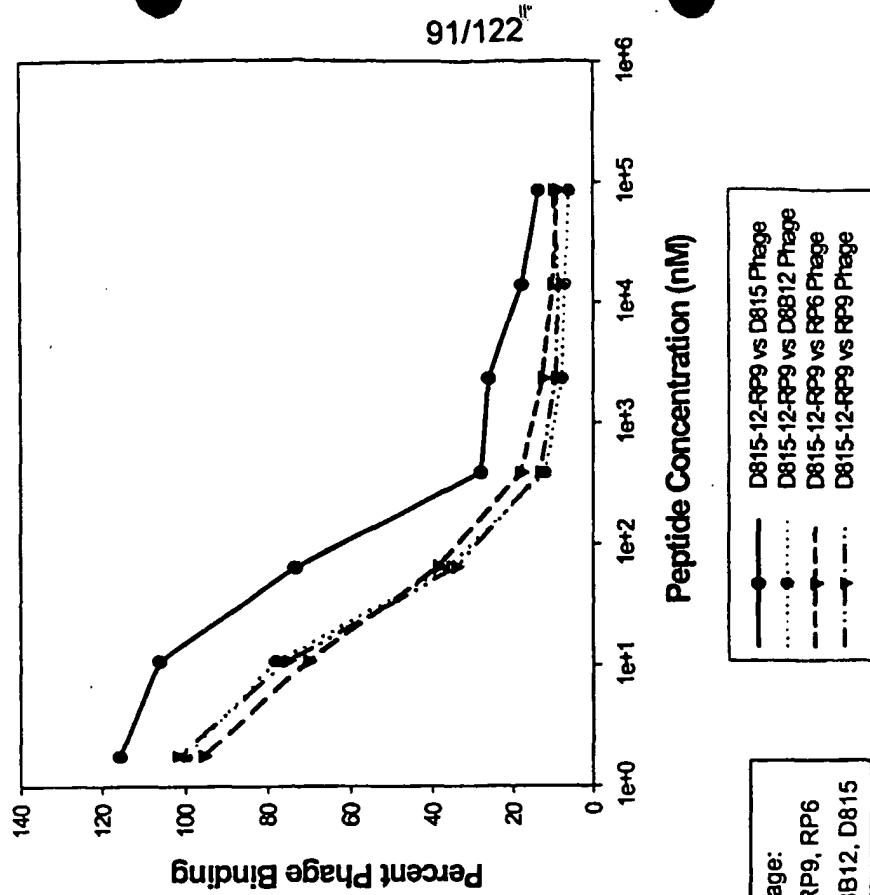
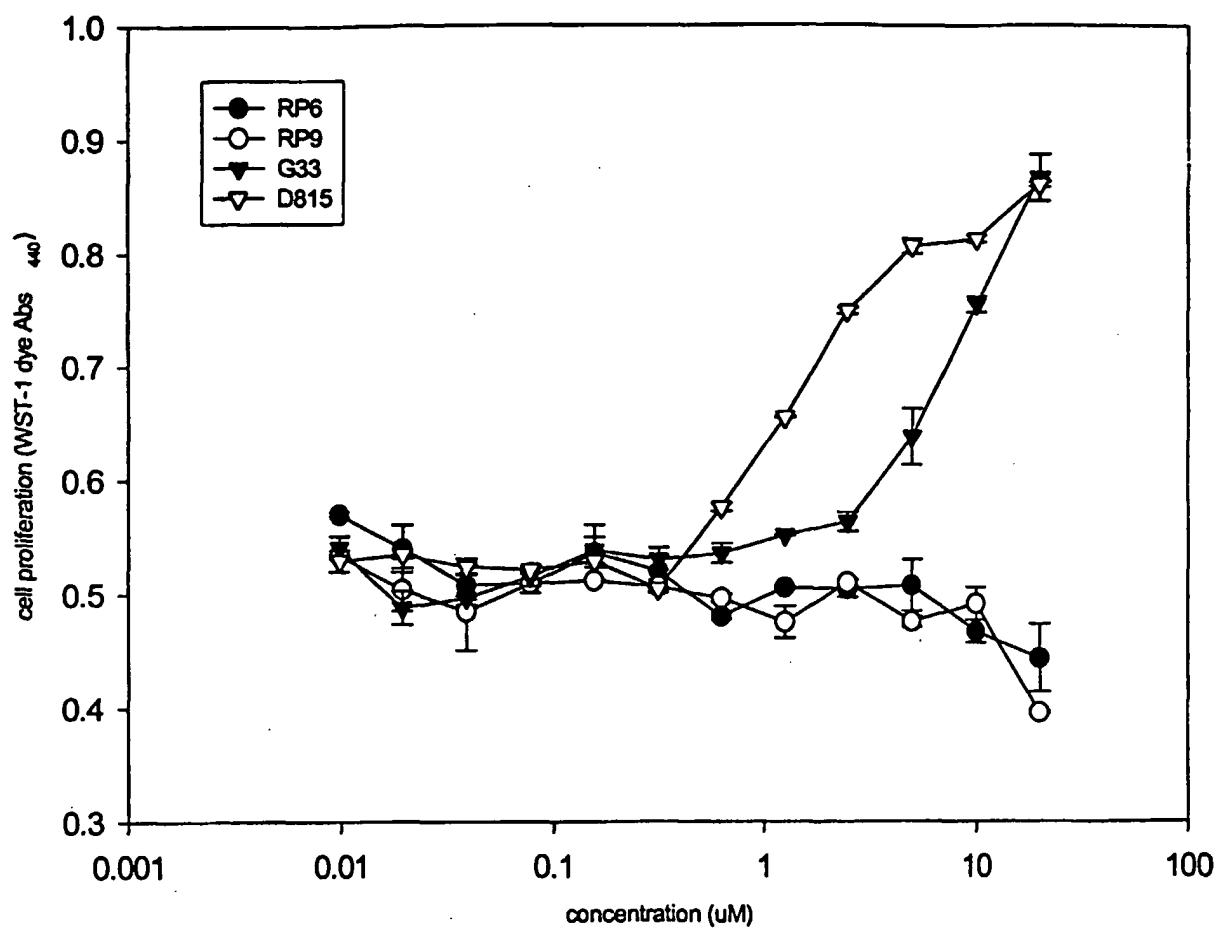
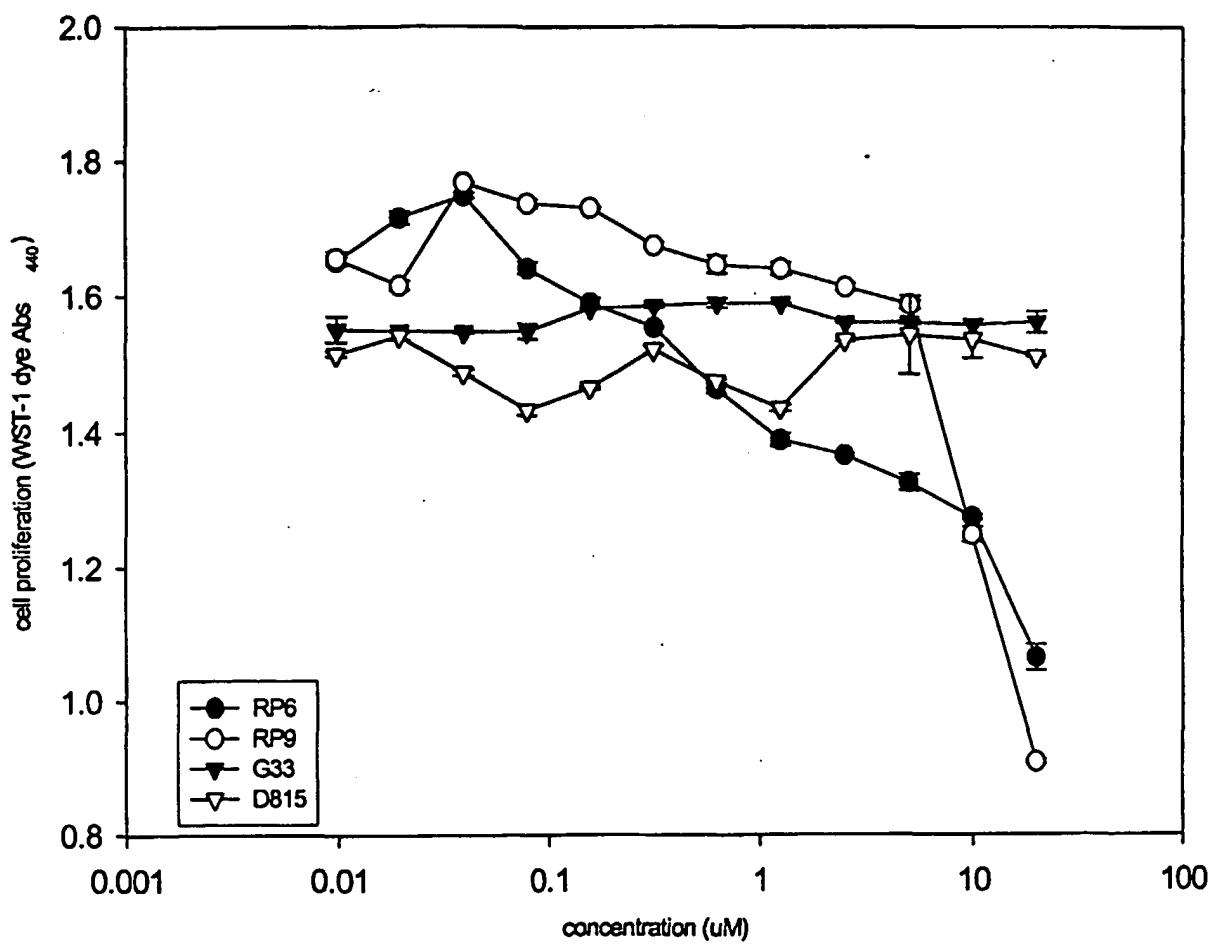


FIG. 35D

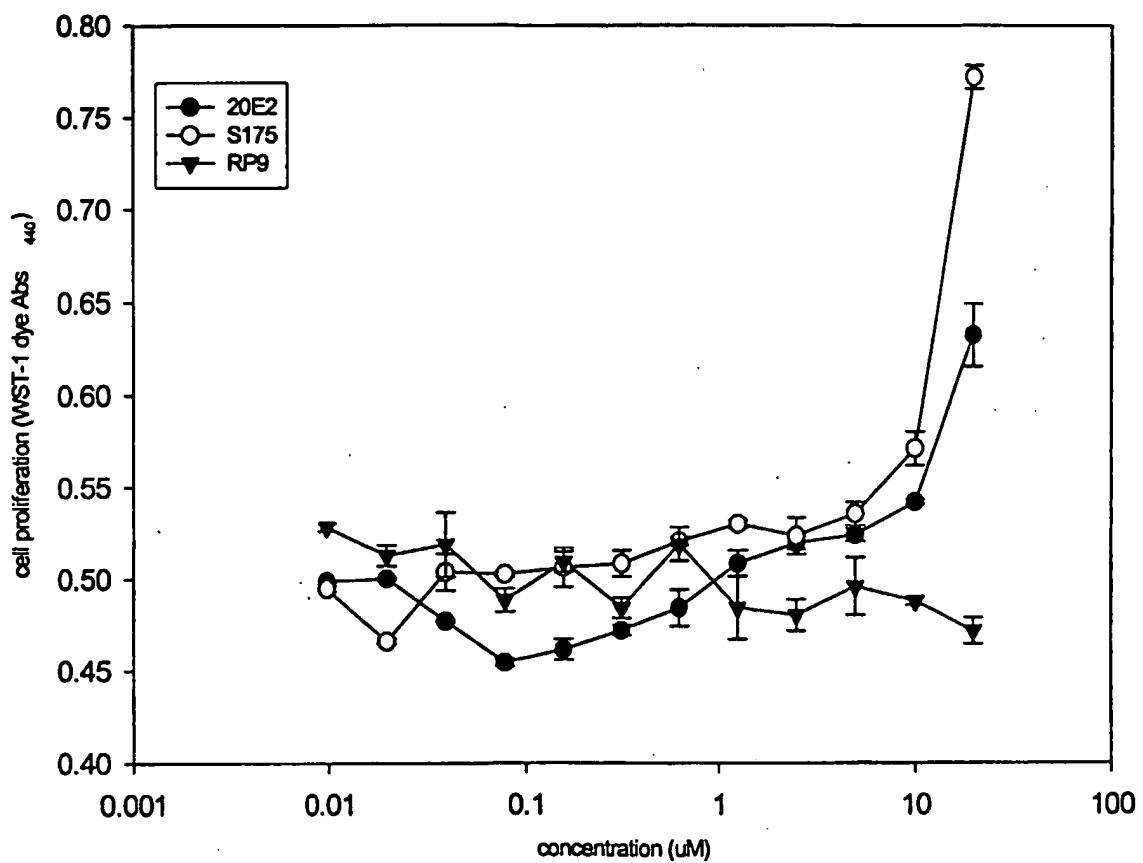
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FIG. 36

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FIG. 37

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FIG. 38

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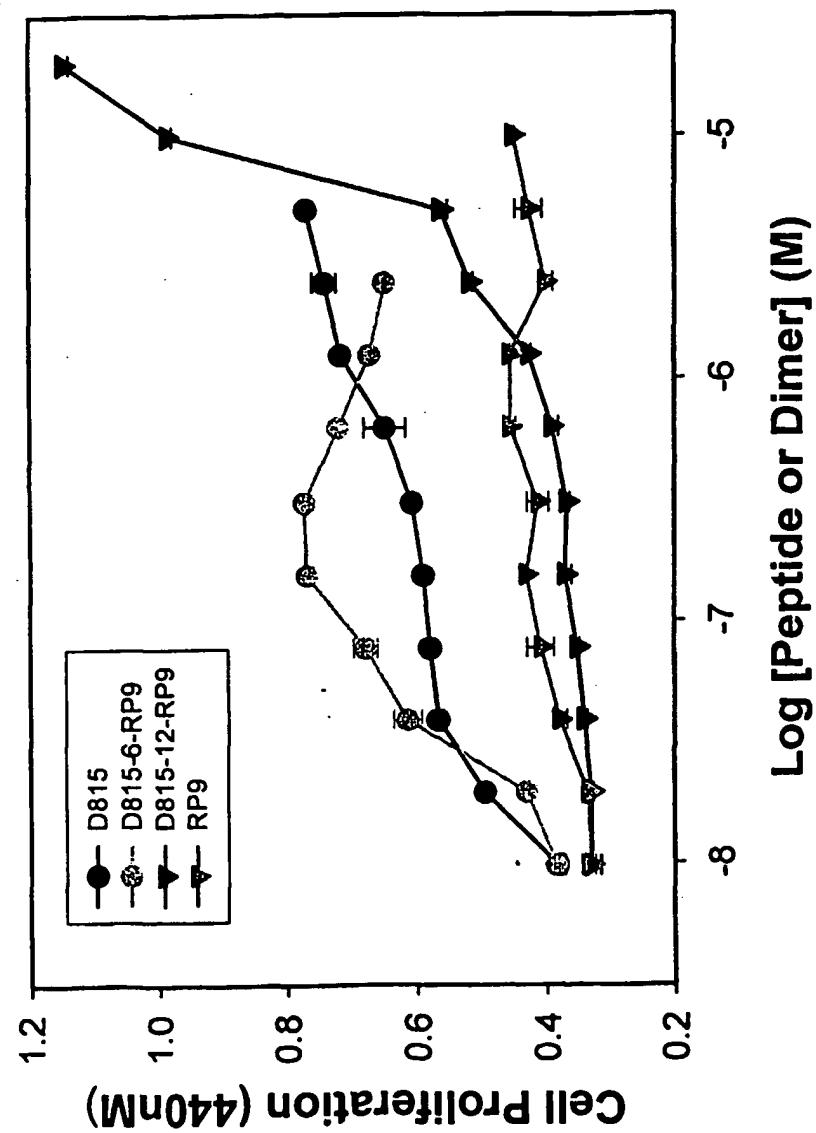


FIG. 39

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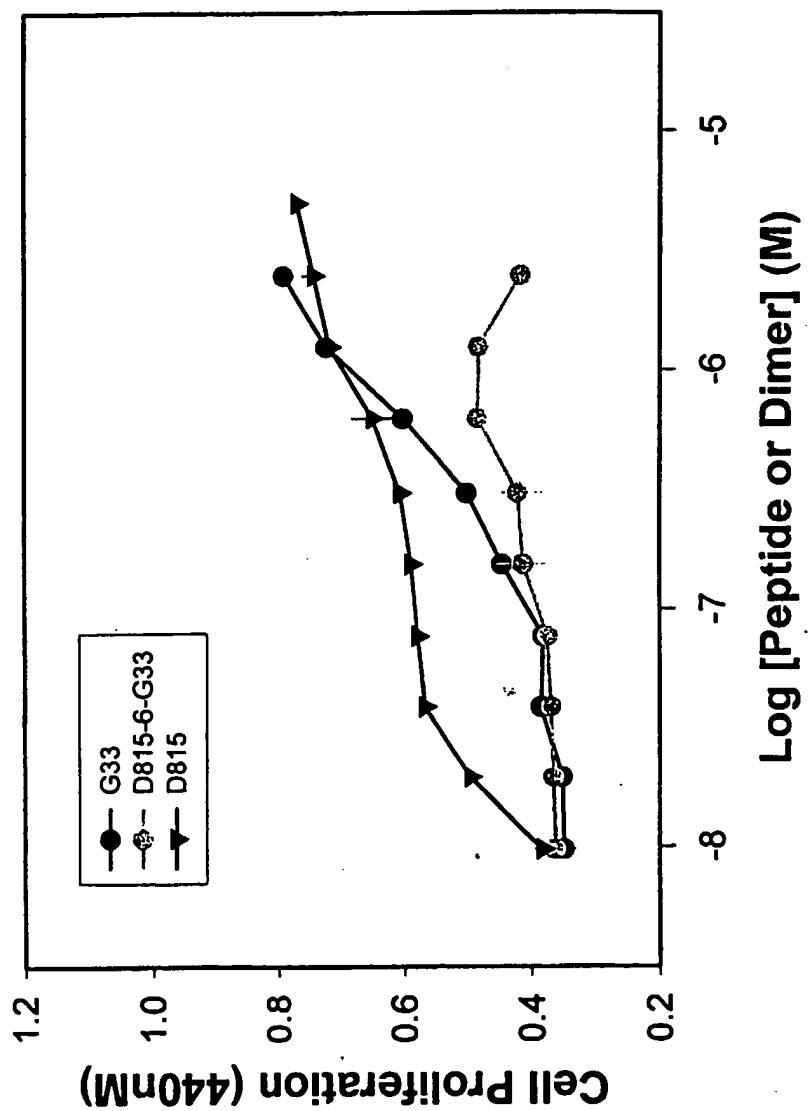


FIG. 40

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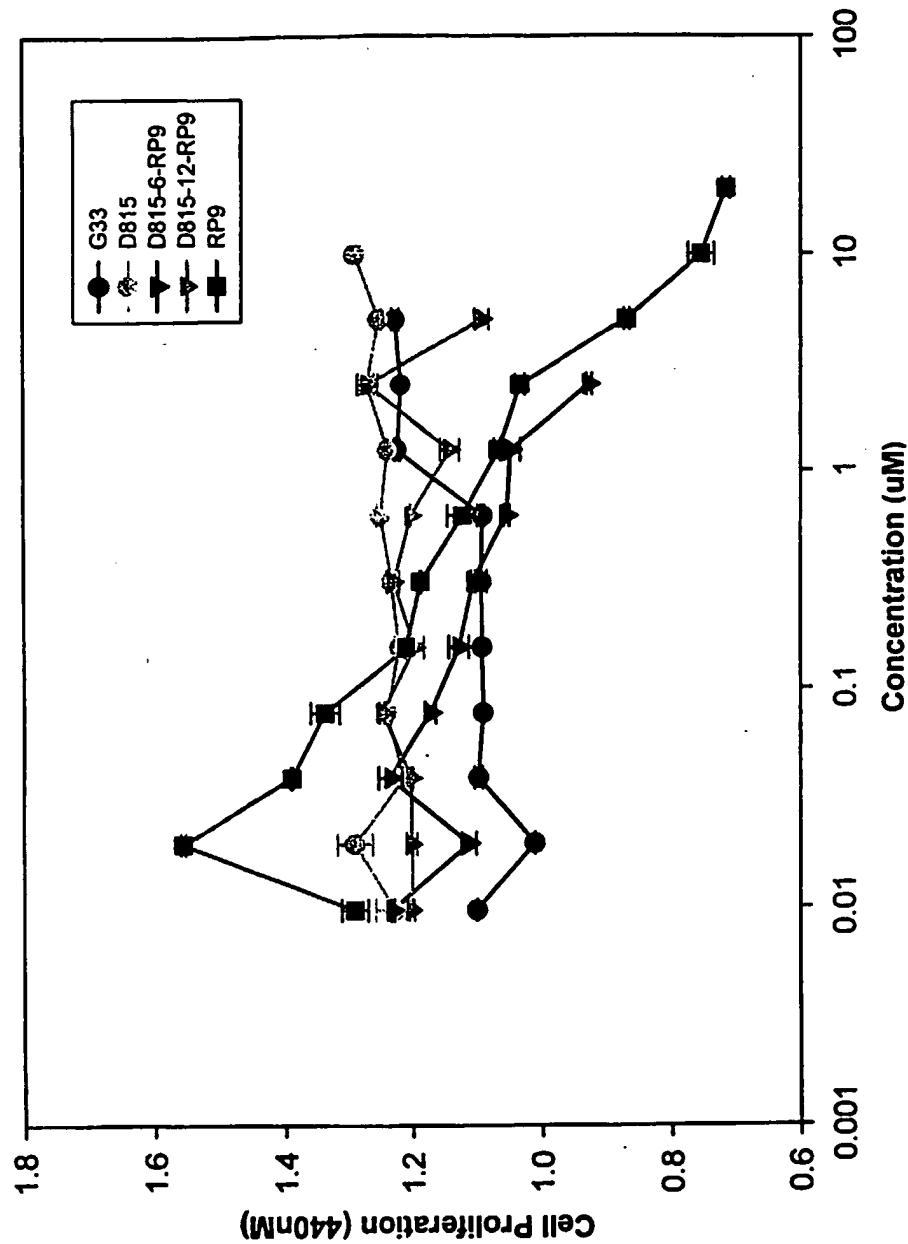
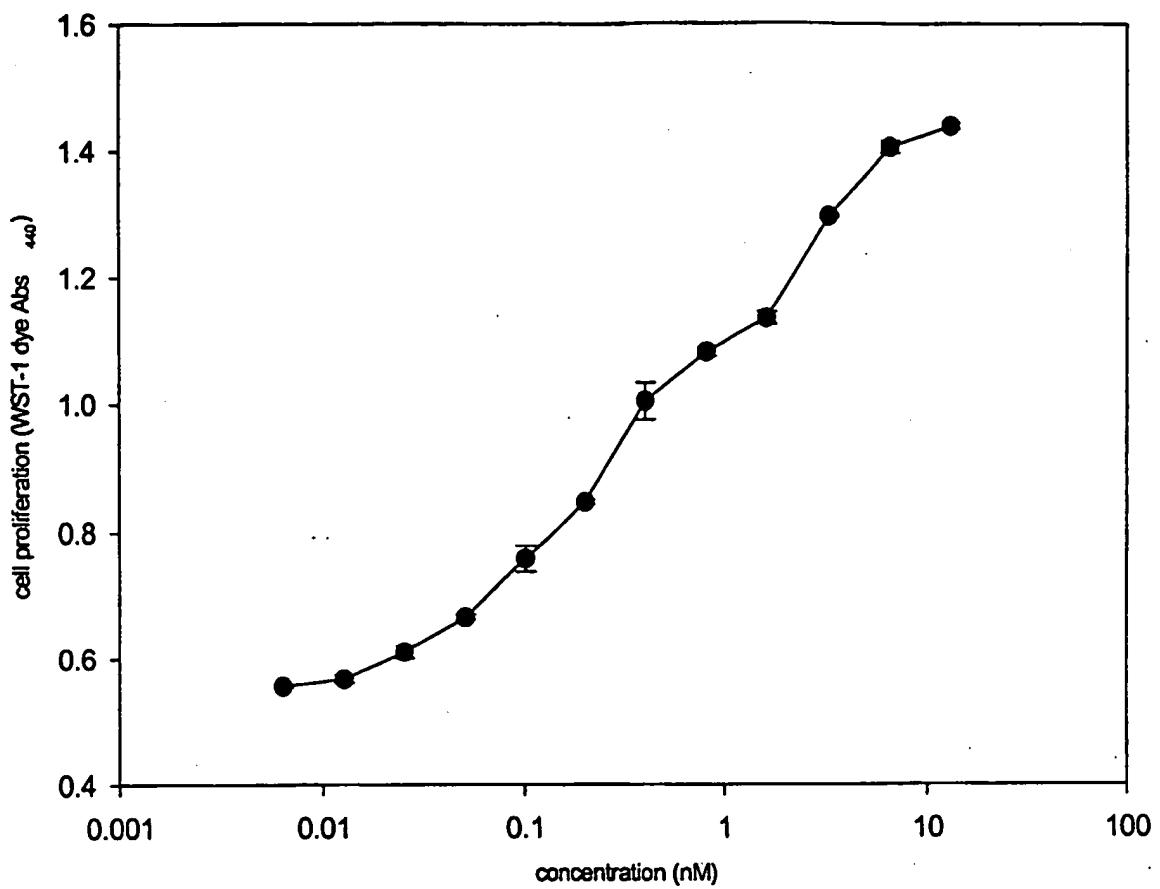


FIG. 41

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FIG. 42

Clone #	Sequence	IGFR	LDH	IGFR/LDH Binder
IGFR-G33-4-A1		15.5	10.3	1.1
IGFR-G33-4-A2		4.8	2.9	2.5
IGFR-G33-4-A3		14.9	16.1	10.6
IGFR-G33-4-A4	PAMAVGYPQPCKSTYERGRSALESRCYQQAAAGAP	8.6	5.4	1.3
IGFR-G33-4-A5		23.5	12.1	3.4
IGFR-G33-4-A6		10.8	5.2	1.2
IGFR-G33-4-A7		13.3	5.0	0.8
IGFR-G33-4-A8	MC PAMACKVC*CCSVSCYDGFPRSGAHPGRWAAGAP	6.0	1.7	1.0
IGFR-G33-4-A9	PAMAFKVSLSCGESFYEWFAGLVRDPTCGWTAAGAP	10.8	6.3	1.1
IGFR-G33-4-A10	MC AGHGACAEFQVMFG*LVHLLGFPGRILGKGLAAGA	6.7	8.9	2.1
IGFR-G33-4-A11	RPWRCGSSLRLVGRRVECYCAERGATRGW*CAAAGAP	5.8	5.8	1.2
IGFR-G33-4-A12	AGHGDGFGLSCKAAVVAVPVQTAGLVRVRAAGAP	3.3	2.6	1.1
IGFR-G33-4-B1	PAMAPRLYQQCPESFYAWTAGHVSPALYGWAAGAP	8.1	3.2	1.2
IGFR-G33-4-B2	PGHGVSVRAGVSQMLRREVAG#CVSPAWEGLGCRCA	4.8	4.1	1.1
IGFR-G33-4-B3	PAMAGMDPQ#CTVASSRWEASPV##VVWRCH#AAAGAP	6.4	2.0	0.9
IGFR-G33-4-B4	PAMAGMFSQTCPEGFYGWWFAQASDSSLCRRAAGAP	5.8	5.4	2.6
IGFR-G33-4-B5	PAMAPLGFFRSCAGAY*VGGGRRVAFE#RCWAAGAP	15.8	2.5	0.9
IGFR-G33-4-B6	PAMAGILCPSCPFLVDS#AAQDAAGQWPSAAAGAP	7.7	2.3	1.2
IGFR-G33-4-B7	MC PAMARRIPRECGDSFYVGLRWLVNPNSDWAAGAP	7.2	3.3	1.2
IGFR-G33-4-B8	PAMADRGVQCPDSFYGWFAVQEPTGTSGLAAGAP	4.8	1.4	1.0
IGFR-G33-4-B9	PAMAGLPS*SCRVAMYKGQAAWSCSAAAGAP	6.2	1.9	1.0
IGFR-G33-4-B10	RPWRLILTVLVEASMTGSGVWYPRRGAGPAEGA	8.5	3.5	1.0
IGFR-G33-4-B11		4.9	3.9	0.9
IGFR-G33-4-B12		3.6	2.6	1.0
IGFR-G33-4-C1		27.7	24.5	1.0
IGFR-G33-4-C2	PAMAGGSARQVQCVTDGVVGWREG*VVDQWL#RAAGAP	28.2	8.4	1.9
IGFR-G33-4-C3	PAMAGIMORACEGGFTDCWLISLISGASSGRAAGAP	29.7	5.3	1.3
IGFR-G33-4-C4	RPWRRVSSLRHVRVTGELFGQQVSELFCLCAAGAP	7.5	5.6	1.1
IGFR-G33-4-C5		4.5	4.4	1.2
IGFR-G33-4-C6	PAMAGLIYMSCLAYFDDLIERRLEKP#RFAAGAP	36.1	22.9	6.3
IGFR-G33-4-C7	PAMAGIMPOSGCGETSGKCMRGQVSILRMRWSAAAGAP	10.0	1.7	1.1
IGFR-G33-4-C8	PAMAFILPRLRSCEDLYDFLASKVWVFRSLAAAGAP	9.7	6.9	1.9
IGFR-G33-4-C9	PAMACMSSQQCPGESFYDWFAQVRDPGWESAAGA	23.3	19.4	9.5
IGFR-G33-4-C10	RPWRCGWAIRGVHRHC*GAWRGQVAQELCR#AAAGA	30.2	9.3	4.3

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FIG. 43A-1

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-C11	PAMAGIASHTCPGGFYEWFAQSRAPGWDGAAAGAP	10.6	6.7	1.1	6.1	HIT
IGFR-G33-4-C12	PAMAGRRIARACPDMSMFGWLAGCGSQOSGWQAAGAP	19.2	30.2	5.2	5.9	HIT
IGFR-G33-4-D1	PAMARPISPLC#RRSKDEDASRVSLLPGFFCAAGAP	2.6	1.8	1.1	1.7	CAND
IGFR-G33-4-D2	MC	6.2	5.1	1.2	4.4	HIT
IGFR-G33-4-D3	PAMADYKDDDKTTFYACLASLMAGTPRQYRTPWARCPAAAGAP	31.0	8.5	1.0	8.3	HIT
IGFR-G33-4-D4	MC	4.8	1.7	1.1	1.5	CAND
IGFR-G33-4-D5	RPWRVNTSESCSL#FVCSLFSGFYECWVGG*WAAGAP	19.5	2.2	1.0	2.2	HIT
IGFR-G33-4-D6	PAMAGMGVQSCHDSFYGWFGLFSDAEGDRAAAGAP	3.4	1.1	1.0	1.1	HIT
IGFR-G33-4-D7	PAMAGDTSRACPESLNG. FCUVGVALARWIAGAP	20.7	15.2	7.0	2.2	HIT
IGFR-G33-4-D8	PAMARWWRLCGERWTHRGWVQVQFPWERGAAGAP	20.1	7.0	1.0	7.1	HIT
IGFR-G33-4-D9	RPWRVPWVLEMPEYGNANLNFYDALQLRLAAAGAP	14.5	6.6	1.7	3.8	HIT
IGFR-G33-4-D10	PAMGRVCYLAGFGEALGGGRVSGFAIQYVRAAAGAP	27.7	19.7	1.2	16.5	HIT
IGFR-G33-4-D11	A GHGV CYLAG FGE ALGG GRV SG FA IQ YV RA AAG AP	29.8	16.9	3.3	5.0	HIT
IGFR-G33-4-D12	PAMAGRRIKEFCRSRSFYDQVACLVKGPSWGGAAGAP	11.0	13.2	2.0	6.5	HIT
IGFR-G33-4-E1	PAMAGRRIKEFCRSRSFYDQVACLVKGPSWGGAAGAP	18.9	16.0	3.7	4.3	HIT
IGFR-G33-4-E2	PAMAGLSSRSACENLRFGRAWQGSDVWDCLAAAGAP	22.4	21.3	0.9	22.9	HIT
IGFR-G33-4-E3	PAMASRIPQWCRLDSFYEWFEQCOLLGPRRESRAAAGAP	14.5	7.3	1.2	6.1	HIT
IGFR-G33-4-E4	PAMAGAESCYRAKSFYDGLGCLVGAEAWWGGAAAGAP	7.8	14.3	1.9	7.4	HIT
IGFR-G33-4-E5	PAMARSAPRCHDPFYEWFAVEAQEPLRCEAAAGAP	6.0	3.1	1.0	3.1	HIT
IGFR-G33-4-E6	PAMAGMGVQSCHDSFYGWFGLFSDAEGDRAAAGAP	13.9	13.9	1.9	7.4	HIT
IGFR-G33-4-E7	PAMADISFESCLAQQLLGWRAGEGSKRLWRCAAGAP	11.9	17.1	3.5	4.9	HIT
IGFR-G33-4-E8	PAMANTFLYPCRDPPFYHSILADLVGVAMOCGAAAGAP	23.2	24.5	5.2	4.7	HIT
IGFR-G33-4-E9	PAMARRIPRECGDGSFYAGLRCLVESPRS DWAAGAP	9.4	5.8	1.7	3.3	HIT
IGFR-G33-4-E10	PAMASIVCPFCEDSFYNWFAAQVADTRGLWAAAGAP	24.1	33.5	10.1	3.3	HIT
IGFR-G33-4-E11	IGFR-G33-4-E12	1.2	0.9	1.0	0.9	
IGFR-G33-4-F1	PAMAWSHSHAYTESYYDWFAAQVLSAGSGRAAAGAP	0.9	1.1	0.9	1.3	
IGFR-G33-4-F2	PAMAGRRIKEFCRSRSFYDQVACLVKGPSWGGAAGAP	7.2	8.5	0.9	9.7	HIT
IGFR-G33-4-F3	PAMARSRRPACGDSFYGWFECVSGLIGRRGAAAGAP	2.2	1.4	1.0	1.4	
IGFR-G33-4-F4	PAMAGISYPACEESFYDCLASLVLSPWGSWGGAAGAP	12.1	5.2	0.8	6.7	HIT
IGFR-G33-4-F5	PAMAGRRIKEFCRSRSFYDQVACLVKGESWNGGAAGAP	16.7	24.2	7.3	3.3	HIT
IGFR-G33-4-F6	PAMAWVAGOYCRDSFYDRLSALVGDAWRCGAAAGAP	13.6	7.4	1.9	3.8	HIT
IGFR-G33-4-F7	PAMACTASRFCAVSYFWFAAQQLPDLGGDSAAAGAP	12.5	16.9	1.2	13.8	HIT
IGFR-G33-4-F8	PAMAGITLQSCCGGFYELLASVVGDTGCRRLAAAGAP	20.2	10.9	1.0	11.3	HIT
IGFR-G33-4-F9	PAMAGYICRSCQGSFYGCCLAAALVRDPRCRSAAGAP	24.7	33.0	8.8	3.7	HIT
IGFR-G33-4-F10	PAMAGRRIKEFCRSRSFYDQVACLVKGPSWGGAAGAP	7.1	10.6	1.2	9.1	HIT
IGFR-G33-4-F11	RPWRVAGAPRCHDPFYEWFAVEAQEPLRCEAAAGAP	1.0	1.0	0.8	1.2	

FIG. 43A-2

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH Binder
IGFR-G33-4-F12	PAMAGMGVQSCCHDSFYGWFGLFSDAEGDRAAGAP	7.6	4.7	0.6	HIT
IGFR-G33-4-G1	PAMASICGQSCRDPFYAGLRLGLLLEPLQLGAAAGAP	17.6	18.5	1.0	19.5
IGFR-G33-4-G2	PAMAGVMSKCCGSFYDWLADLVEAISWSWAAGAP	6.5	5.7	1.0	5.5
IGFR-G33-4-G3	PAMASFSGEACGGSFYDCLAGLMRDSSVSRAAAGAP	18.4	7.9	1.1	7.4
IGFR-G33-4-G4	PAMASFSTCMETLLDGFFGQAFNRCRRTAAAGAP	22.5	20.1	1.3	15.6
IGFR-G33-4-G5	PAMARVIVYPTCPRDFYGGIAALVFGPHVCRGAAAGAP	22.8	21.7	1.9	11.5
IGFR-G33-4-G6	PAMAGIGSQACTDPFYWFEGLVSNNGWCRRAAGAP	5.9	5.3	1.2	4.3
IGFR-G33-4-G7	PAMAGRRIKEFCRSRSFYDQVACLVKGPWSWGGAAAGAP	18.8	2.1	1.0	2.1
IGFR-G33-4-G8	PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP	23.6	30.3	3.7	8.2
IGFR-G33-4-G9	PAMADMMSQVCSQSMTGRFSVDFYDGLRCLLAAGAP	17.3	4.6	0.9	5.1
IGFR-G33-4-G10	PAMARRIPRECGDSFYAGLRCLVESPRSDWAAGAP	26.8	24.6	5.4	4.6
IGFR-G33-4-G11	PAMARVIOQEACGGSFYDGLIACLVYPQGWRRGAAGAP	3.3	1.5	0.9	1.7
IGFR-G33-4-G12	PAMAGGRSVACQESFYALLGCVVVMGEGGSSAAAGAP	24.1	32.1	12.1	2.7
IGFR-G33-4-H1	PAMAGISFRSCLQLIAQSAGNASEMIGCRSAAGAP	5.9	5.8	1.2	4.8
IGFR-G33-4-H2	PAMAGIGRDSYCQGAFYDWAGLVDGGLFCQAAGAP	9.2	4.4	1.0	4.4
IGFR-G33-4-H3	PAMAGISYQSCEDSFYAWFACTVLDTRGGGAAGAP	17.8	16.0	1.8	8.9
IGFR-G33-4-H4	PAMARVIEACGGSFYDGLIACLVYPQGWRRGAAGAP	3.1	3.2	1.1	2.8
IGFR-G33-4-H5	PAMADMPLLECLDPFYSWEAGQVSDERFCGAAAGAP	20.1	7.5	0.9	8.0
IGFR-G33-4-H6	PAMARVIOQEACGGSFYDGLIACLVYPQGWRRGAAGAP	5.1	2.4	0.8	2.9
IGFR-G33-4-H7	PAMAGRRIKEFCRSRSFYDQVACLVKGPWSWGGAAAGAP	12.9	11.1	1.1	9.8
IGFR-G33-4-H8	MC	23.4	23.5	1.6	14.7
IGFR-G33-4-H9	PAMAHISFHSCLEALQDPENGQPSAAWRNCAAGAP	1.2	1.1	0.8	1.3
IGFR-G33-4-H10	PAMAMTAQESCPDSFYECIABLVLGVDRWGGWAAGAP	7.9	10.4	2.8	3.7
IGFR-G33-4-H11	PAMAHISFHSCLEALQDPENGQPSAAWRNCAAGAP	16.8	23.7	1.3	18.1
IGFR-G33-4-H12	PAMAGTISQCCEENFYAGLALLAGVGQONGCAAAGAP	20.4	19.0	4.0	HIT

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FIG. 43A-3

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Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
B10	DDDKTFYACLEFLISGNPEGNSCPWDRCR	2.6	29.0	1.0	29.0
D1	MC	17.0	26.8	1.1	HIT
A4	DDDKTFYSCLASLLHGGPQRNTGPWERCR	25.2	25.6	1.0	HIT
A6	DDDKTFYSCLASLLTGPREQNRGAWERCR	22.3	23.3	1.1	HIT
B1	DDDKSFYSCLASLLTASRLPSRGAWDGCH	18.0	22.8	0.9	HIT
E4	DDDKSFYSCLGSLLTGAPQPIRGAWDRCR	20.8	20.6	1.1	HIT
C11	DDDKSFYSCLASLWSGTGGSSRGRWEGCR	22.7	20.4	1.3	HIT
C2	DDDKTFYSCLGALLAGTGERNLRPWGRCR	19.9	19.1	1.0	HIT
B6	DDDKTFYSCLGSLUTGPSPDKRGPGGCCR	22.6	19.0	1.2	HIT
A12	DDDKTFYKCLASVLTGTSTOTKRPWEGCR	13.9	18.8	1.0	HIT
D2	DDDKPFYSCLATLTLTDPQSQRGAWRGRCR	22.5	17.2	1.1	HIT
C1	DDDKSFYSCLAALLTGSQSQQGGAWMLCR	21.6	16.9	1.1	HIT
F10	DDDKSFYSCLASLIVAGTPWPKGGSWERCR	11.4	16.6	0.9	HIT
B11	DDDKSFYSCLASLVTTGIPRSNSGTTQVFCR	7.8	16.1	0.8	HIT
A9	DDDKSFYSCMASLTLTGTPESRGMGERCG	16.3	15.9	1.3	HIT
A10	DDDKAFYSCLASLUTGSPPAQGGPWDRCR	8.3	15.6	1.0	HIT
B9	DDDKFFYSCLASLSDTPQRQRGPGVRCR	5.9	14.3	0.9	HIT
A3	DDDKTFYSCLASLLAGNPQPNRAGWEYCR	14.1	13.3	0.9	HIT
F2	DDDKTFYSCLGSLLGPQKNPGGERCR	9.6	13.3	0.9	HIT
A7	DDDKMVFYSCLESLLIGRMWRNGGSSLSCR	10.9	13.2	1.1	HIT
G3	DDDKSFYSCLTFLTGTPOQANDAWERCR	9.6	13.2	1.0	HIT
A11	DDDKSFYSCMAALLSGAPQKSRRWERCG	4.7	12.7	0.8	HIT
B7	DDDKAFYRCLAYLLAGRPOASGGGVYCR	19.7	12.5	0.9	HIT
D5	DDDKAFYSCLAALRERSPQMSRGTWGCCR	21.8	11.8	1.3	HIT
E8	DDDKTFYACLAALLGGTAELHDGSLBCRG	11.8	11.8	1.3	HIT
D10	DDDKTFYSCLGSLLTGTLPMPARGARNICR	15.1	11.6	1.0	HIT
D8	DDDKTFYSCLSSLAGSPLPRRDWLWAGCR	11.1	9.2	0.9	HIT
D12	DDDKAFYSCMASLLAGTPEAQGSAWVCR	5.4	7.4	0.8	HIT
A5	DDDKNFYACMESLVSAPPSSRDPFECRR	16.0	7.3	1.3	HIT
F6	DDDKSFYSCLASLVSGETA.PNRGPWERCR	4.2	6.9	1.0	HIT
G5	DDDKIFYSCLASLDDTAQSRRGQWARCR	4.8	6.4	1.3	HIT
C6	DDDKIFYSCLGALLSGTIPQTSHVTSGRCR	13.6	5.9	1.1	HIT

FIG. 43B-1

Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
D3	TDDKTFYFCLASLLAGTQQQSRGAWEWRCG	5.1	5.9	0.9	HIT
A2	DDDKAFYSCLASVLTGSPHPGRSRWERCR	18.6	5.5	0.9	HIT
B2	DDDKTFYSCLESMLTGTTPPCRGHGERGR	8.0	5.5	1.0	HIT
D7	DDDKTFESECLEALVSGSRRERGLWYRCR	10.6	5.5	1.1	HIT
C12	DDDKAFYSCSSLLAGTRERHDTWPRCG	12.0	5.3	1.0	HIT
B5	DDDKTFHSCLAALVLTGTPQQKRGPWCR	20.0	5.1	1.3	HIT
E2	DDDKTFYLCLASLQTVTTRLGDRVPWCR	18.0	4.6	0.7	HIT
F3	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	3.7	4.5	1.4	HIT
H2	DDDKSFYSCLASLNCTPGLLRCQWERCR	7.2	4.4	0.8	HIT
B4	DDDKTFYCSLSSLLASSTPQPNRGAWLCCR	11.9	4.3	1.2	HIT
G12	DDDKSFYSCLASLNCTPGLLRCQWERCR	2.2	4.2	1.0	HIT
F5	MC	8.4	4.0	0.9	HIT
A1	DDDKTFYSCLGALLSGAPQTYRGAGCR	8.1	3.9	1.0	HIT
E11	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	4.9	3.8	1.0	HIT
F9	DDDKHFYSCSSLTAPPQSTRGPAGRHC	3.9	3.7	1.0	HIT
B3	DDDKTFYSCLASLNLNTOPNGQWVRCR	1.7	3.6	0.8	HIT
G4	DDDKVFYTCLASLSTGTQROSQEWRQR	9.4	3.3	1.3	HIT
E5	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	4.6	3.2	0.9	HIT
C10	DDDKPFYSCLASLIQQTPLPERGMWERCR	6.3	3.0	1.0	HIT
C7	MC	10.6	2.9	1.0	HIT
H5	DDDKTFYSCSVSLLTGAQRDGVWBRCR	5.5	2.9	1.3	HIT
H3	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	4.6	2.8	1.1	HIT
C3	DDDKAFYGCCLAALLTGAROPSRGVGERCFC	6.0	2.7	1.5	HIT
F1	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	1.5	2.6	0.8	HIT
B3	DDDKTFYSCLASLTLAGSPQPKRAGWEYCR	8.6	2.5	1.0	HIT
D11	DDDKPFYSCLESLVTCRPOADRGWWERCR	4.9	2.4	0.9	HIT
E10	DDDKTFYSCLTLSRSRCAHGLSGRWERCR	5.0	2.3	0.9	HIT
F12	DDDDKTFYFCFLATLTLTGPVPNREPWACR	2.5	2.2	0.8	HIT
D4	DDDKFIFYSCLRLTGTNPPEPVRGPFDRCG	3.0	2.1	1.1	HIT
E6	DDDKTFYSCLVSLNLNTAKPNRGQWEGCR	2.7	2.1	1.4	HIT
F11	NDDKSFYSCVASYLVNEGPSQVGVLGERCR	3.5	2.1	1.2	HIT
A8	DDDKTFYSCLASMLTGTGPPHPDWPDRCR	8.3	2.0	1.0	HIT
F7	DDDKKFYSCCLVELVNGTSPPARGLWERCR	2.7	2.0	1.0	HIT
F8	DDDKVFYSCLESLVSGTPEVNGRRAWERCR	2.0	1.8	1.0	CAND
E12	YDDDKRFYFCLASLASLQTNREQWERCR	1.0	1.7	1.0	CAND
H1	DDDKTFYTCLESLLNLNTPQRNRGQWDPCS	1.9	1.7	0.9	CAND
H8	DDDKTFYTCLESLLNLNTYERPGGRWBESCR	1.2	1.7	1.2	CAND

FIG. 43B-2

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Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
G9	DDDKAFYSCLATLLYGNPPSSRGQWHRCR	1.5	1.6	1.1	CAND
D6	DDDKVFYSCLESLVSgtPEVNGRAWERC	1.3	1.5	1.1	CAND
H6	DDDKTFYACISSLILYGTADWSQGQRDRCR	3.2	1.5	1.3	1.1
E7	DDDKSFYSCLMESLWTTDPQPNRGRWERCR	3.2	1.4	1.1	1.3
F4	DDDKTFYSCASLLTVSPEPSRGWERCR	1.4	1.4	1.2	1.1
G6	MC	1.4	1.4	1.0	1.5
G11	DDDKTFYSCMVQLLTGTPEKSCVTWERWR	1.6	1.4	1.0	1.4
H4	DDDKTFYSCLVSSLNGTAKPNRGQWEGCR	4.7	1.4	1.0	1.4
B8	DDDKTFYSCVASLUVGTAAQPQCGPWQGWG	9.1	1.3	1.0	1.3
C8	DDDKTFYSCLAGLVTGPPRQNMGAGDACR	7.7	1.3	1.4	1.0
E1	DDDKTFYSCMSSLSTTAPOPKSGRWDRCR	2.3	1.3	0.9	1.4
G10	DDDKTFYSCLASLIVNGSLQPNRPGELCR	1.2	1.3	1.2	1.2
H10	DDDKTFYSCLVSSLNGTAKPNRGQWEGCR	3.5	1.3	1.0	1.3
H11	DDDKTFYSCLVSSLNGTAKPNRGQWEGCR	2.6	1.3	1.0	1.3
D9	DDDKSFYSCLESLVLNGGPQQKRGPGWEGCR	1.1	1.2	0.9	1.3
E9	DDDKTFYSCASLLNLNTQPNGGQWVRCR	1.1	1.2	1.1	1.1
C9	MC6	1	1.1	1.1	1.0
G1	DDDKSFYSCLASLSNCTPGLLRCQWERCR	0.9	1.1	0.9	1.3
H7	DDDKNFYSCSALLNGNTVSDRGQGERCF	2.2	1.1	0.9	1.1
H9	DDDTFYSCLASLIVNGSPQAQYGGPREHCR	3.1	1.1	1.1	1.0
C5	DDDKTFYSCLAWLMTGPAPPYRGWSCWS	3.3	1.0	1.0	0.9
G7	DDDKTFYACSSLSTGTWQPKRGPGERCR	0.9	1.0	0.9	1.1
H12	DDDKSFYSCLASLSNCTPGLLRCQWERCR	3.7	1.0	1.0	1.1
C4	DDDKTFYSCLGSLINGTPPPHRGLWQGCR	1.1	0.9	0.9	1.0
G8	DDDKTFYSCLVSSLNGTAKPNRGQWEGCR	0.7	0.9	1.0	0.9
G2	DDDKQFYSCLAFLASGLIAQPPGAWECLR	1.1	0.8	1.0	0.8
B12	DDDKTFYCCCLAALLTGAPPKGTCERCG	9.3	13.2	0.7	18.3

FIG. 43B-3

<u>IR</u>	Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
	B5	FHENFYDWFAKDKDGGSGGSGGSDILCVLLEELFWGDSSLFDYCTG		17.0	16.9	0.5	35.8
	A3	FHENF .DWFRQVSGGSGGSGGSGGSNLCVLEELFWGASLFGECSCG		13.0	11.8	0.3	35.8
	A8	SHGNFSEWFVRQGYGGSGGSGGSGSNSLFCVG		13.2	13.1	0.4	33.2
	C7	FOESFYDWFVFR .VTGGSGGSGGSGGSDILCGVEDLWNGSALS GYCA G		15.1	14.7	0.5	30.6
	B4	FHENFNDWFWFREVSGGSGGSGGSDILCVLLEELFWGASLFSYCSG		13.2	11.7	0.4	27.6
	B1.1	SHENFYDWFVFR .GPGGSGGSGGSGGSHLCVLEELFWGDSLFGACPG		10.9	9.1	0.3	27.0
	A9	FHENFYDWFWARQVSGGSGGSGGSGGSHLCVLEELFWGASLFA .CSD		10.7	12.3	0.5	25.7
	A6	FPDNFYDWFWFVFR .VSGGSGGSGGSGGSGGSHLCVLEELFWGASPF GYCSG		11.6	8.7	0.4	19.8
	A4	FQENFYDWFWGRQISGGSGGSGGSPLCVDVEELFWGVSLFGCYCTG		13.6	12.1	2.6	4.6
	C8	FQENFYDWFWFVFR .ASGGSGGSGGSGHLCVLEELFWGSSQFRYCSG		16.0	14.5	3.2	4.5
	A10	FHENFYDWFWARQVYGGSGGSGGSHLCVLEELF .GASLFATCSD		10.6	6.0	1.5	3.9
	D1.1	FHENFYDRIVRQVAGGSGGSGGSGGSGGSGSSRLCDLEELFWGASL .GHC S G		12.4	5.5	1.5	3.6
	D4	FHKNFYDWFWFDRQVSGGSGGSGGSGGSGSSRLCDLEELFWGASL .GHC S G		15.4	9.8	3.9	2.5
	C1	FHENFYDWFWFIRQDGGSGGSGGSGHLCVLEELGGASPF GYCSG		16.8	2.7	1.3	2.1
	D1.2			11.7	8.7	4.6	1.9
	D8	SNENFYDWFWFDRQVSGGSGGSGGSHLCVLEELSWGASLFGCYCG		15.8	9.6	7.4	1.3
	C11	FHESTFYDWFWFDRQVSGGSGGSGGSHLCVLEELWGASVFGCCSG		11.0	5.8	5.4	1.1
	C4	FHETFYDWFWFDR .VSGGSGGSGGSGEELFGGASLFGYPSG		16.7	13.2	15.0	0.9
	D1	SHENFYDWFWFDRQVSGGSGGSGGSGSNSLCDLDEVS .GASLGCGYRSG		16.2	5.5	7.1	0.8
	C6	FH .NFYDWFWFCQVPEWIPMTIHAVLTCAVLEEPITWGDSSLFGY G .E		16.1	1.7	2.2	0.8
	A5	SHENFYDWFWFQRV .GGSGGSGGSGGSHLCVLEELGGASL MGSCSG		16.0	8.7	12.9	0.7
	B8	SHENFYDWFWFV .VSGGAAAGAPPAMASHENFYDWFWFVR .VSGG		15.2	8.9	13.9	0.6
	D2	FHENFYDWFWFIR .VGGGSGGSGGSDILCVLEDDCSRAAGAP		13.9	8.4	13.1	0.6
	A2	DYKDASVSGTFHDAYEWFWR .VGS		13.4	6.9	12.6	0.6
	C1.2	FHENFYDLVLPSSAGSWIRWLWRF .PVRLGRTVILGCFSDR .LFW		9.2	4.4	6.8	0.6
	B9	FHENFYDWFWFDRQVSGGSGGSGGSQLCDL .EVIWGASLFGYCTG		17.8	8.2	16.3	0.5
	B1	VHENFYDWFWFDRQVSGGSGGSGGSQLCDL .EVIWGASLFGYCTG		18.1	13.4	0.5	1.8

FIG. 44A-1

Clone	Sequence	IGFR	IR/IGFR	IGFR/IR
B2	FHENFYDWFDR . VSGCGGGSHLCVPEEQFWGGASRFGYCSA	16.2	7.0	1.9
D9	FHDNFYDWFVRQVSQGGGGSRQCQGASL . GYCSG	17.0	6.6	2.1
D5	OUT OF FRAME	16.1	4.8	2.2
C9	FHEDFYDWFVFR . VPAGGGGGSHLCVPGYCSS	17.2	5.1	2.8
B7	FRENFYDWFVFC . VSGGGGGGGNLCVLEAAAGAP	15.9	4.0	2.7
D6	OUT OF FRAME	15.6	4.2	3.0
C5	GHDNFYDWFVFRQVSQGGGGSHLCV . GAFPWGYCSD	15.2	3.6	3.0
D3	FH . NFYDWFVFRQVYGGGGSGTAAAGAP	16.2	3.5	3.4
C10	BAD SEQUENCE	11.2	2.5	3.1
A7	FHENFYDWFGRQVYGGGGSPVCILGELS . GGALFGDCSG	15.5	1.8	2.9
A12	FHENFYDWFVFR . LSGGGGGGGSHLCVPEERLWGDPLFGYCSG	8.7	1.2	3.0
D7	FH . NFYDWFVFRQVTGGGGGGGGSHPAR	16.2	3.0	4.0
A11	FHENFYDWFVFRQVTGGGGGGGGSHLCVLEELS . GAALPGYCSG	11.8	1.0	4.1
D10	VQGSFYDWFVFRQVSGGGGGGGSHLC . GSG	12.7	1.0	6.6
C3	FHENFYEWFVFRQVSQGGGGGGGGSHRCDEELH . CASG	16.8	0.6	4.2
A1	DYKDGTYWGSFYEGLM . LVQSGTSG	13.6	1.7	7.1
B6	OUT OF FRAME	12.7	1.0	8.1
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Non-Binders:				
B12	FHENFYDWFDRQVSGGGGGGGSHRCVLEERFWGASLFG . CSG	7.8	0.5	1.6
B10	SHENFYDWFVHQVSGGGGGGGSHLCVLEERF . GPSLFGYCSCG	10.8	0.6	1.4
B3	FHANFYDWFRRQVSGGGGGGGSHLCVLDLQDMF . GGSGAAAGAP	16.9	0.7	1.2
C2	FODNFYDWFVFRQISGGGGGGGGSHLCVLESWF . GASLFGYCSCG	14.8	0.5	0.6

Non-Binders:

- | | | | | | | |
|-----|---|------|-----|-----|-----|-----|
| B12 | FHENFYDWFDRQVSQGGSGGGSHRCVLEERFWGASLFG. CSG | 7.8 | 0.5 | 1.6 | 0.3 | 3.3 |
| B10 | SHENFYDWFVHQVSQGGSGGGSHLCVLEERF. GPSLFGYCSCG | 10.8 | 0.6 | 1.4 | 0.4 | 2.3 |
| B3 | FHANFYDWFFFRQVSQGGSGGGSHLCVLFQDMF. GGSGAAAGAP | 16.9 | 0.7 | 1.2 | 0.6 | 1.7 |
| C2 | FODDNFYDWFVFRQISGGSGGGSHLCVLESWF. GASLFGYCSCG | 14.8 | 0.5 | 0.8 | 0.6 | 1.7 |

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FIG. 44A-2

<u>IGFR</u>	Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
H11	FHETFYD.L	<u>G</u> R <u>L</u> V <u>F</u> GG <u>S</u> GG <u>S</u> GG <u>S</u> HH <u>L</u> <u>C</u> V <u>P</u> EE <u>L</u> FW <u>G</u> T <u>S</u> LL <u>S</u> Y <u>C</u> SG	9.3	0.4	4.0	0.1	11.4
F11	FHENFYDWFVFRQVSGGSSGGSSHHL.GSG	12.5	0.8	5.2	0.2	6.5	
E2	FHENFYDWFVFRQVSGGSSGGSSHH <u>R</u> <u>C</u> GLEEPV.GASLV <u>G</u> <u>Y</u> <u>C</u> AG	13.4	1.3	7.5	0.2	5.7	
G7	FHANFYDWFVFRQV.GGSSGGSSGGSSHH <u>L</u> <u>C</u> V <u>R</u> EE <u>E</u> LF.GASLL <u>G</u> <u>D</u> <u>C</u> SG	16.1	2.1	9.8	0.2	4.7	
G12	FHEDFYDWFVFRQVGGGGGGGGSHL.CVREELF.GASLL <u>G</u> <u>D</u> <u>C</u> SG	9.4	1.2	5.5	0.2	4.6	
H7	.HENFYDWFVFRQLSGGGGGSDGSHL.FCYGSG	7.2	0.6	2.7	0.2	4.5	
G11	OUT OF FRAME		11.4	1.4	5.8	0.2	4.3
F7	FHENFYDWFDRQVGGGGGGFSPV <u>R</u> T <u>G</u> RTV <u>L</u> GG <u>F</u> SV <u>R</u> LL <u>W</u>	15.0	2.7	10.9	0.2	4.1	
G1	SHDNFYDWFVFR.VSGGSSGGSSPL <u>C</u> VL <u>G</u> N <u>C</u> SG	11.3	2.8	10.6	0.3	3.8	
E8	FYDNFYHWFD.R.VSGGSSGGSSHH <u>L</u> <u>C</u> V <u>E</u> ER <u>V</u> <u>C</u> GA <u>S</u> LF <u>D</u> <u>Y</u> <u>R</u> SG	13.5	0.9	3.4	0.3	3.7	
E9	FSEHFYDWFARQVSGGGGGGGSHL.CVLDERF.GASM <u>V</u> <u>G</u> <u>C</u> SG	14.5	0.7	2.3	0.3	3.6	
G2	FPENFYDWFDRQVSGGGGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5	
E3	FHENFYDWFDRQVSGGGGGGGSHQ <u>C</u> QE <u>E</u> RF <u>W</u> GA <u>S</u> L <u>C</u> GY <u>C</u> SG	15.9	1.9	6.7	0.3	3.5	
E12	FHDSSFYDWFVFRQVSGGGGGGGSHL.CVGLEELF.GASRF <u>G</u> <u>D</u> <u>C</u> SG	10.0	2.3	6.8	0.3	2.9	
E5	OUT OF FRAME		14.7	3.7	9.6	0.4	2.6
F8	FHGDFYDWFVFR.VSGGSSGGSSH <u>L</u> <u>C</u> V <u>E</u> EL <u>Y</u> <u>C</u> SG	13.7	3.6	9.5	0.4	2.6	
E6	FHDNFYDWFVFR.VSGGSSGGSSH <u>L</u> <u>C</u> V <u>E</u> ER <u>F</u> WG <u>G</u> <u>S</u> <u>P</u> <u>I</u> <u>G</u> <u>Y</u> <u>C</u> SG	13.3	3.0	7.3	0.4	2.5	
G8	OUT OF FRAME		13.9	4.5	10.5	0.4	2.4
E1	FQDNFYDWFVFRQVSGGGGGSSH <u>R</u> <u>C</u> TV <u>E</u> LG <u>C</u> SG	13.4	5.8	13.3	0.4	2.3	
H12	FHENFYDWFDRQVSGGS <u>A</u> <u>C</u> LF <u>G</u> <u>C</u> SG	9.8	3.9	8.5	0.5	2.2	
F2	YHENFYDWFVFR.VSGGSG	14.4	6.2	12.8	0.5	2.1	
H6	VHESFYDWFVFR.VAGGSSGGSSGGSHL.CDV <u>D</u> <u>C</u> SG	11.5	4.8	9.6	0.5	2.0	
H4	FHDNFYDWFDRQVSGGGGGSSLL.CVGEEPF <u>W</u> GA <u>S</u> L <u>F</u> A <u>Y</u> <u>C</u> SG	11.2	5.3	10.0	0.5	1.9	
H5	FH.HFYDWFDRQVSGGGGGSSLL.CVGEEPF <u>W</u> GA <u>S</u> L <u>F</u> A <u>Y</u> <u>C</u> SG	11.8	4.4	8.5	0.5	1.9	
E7	FHENFYDWFVFRQVSGGGGGGGGGGG	15.4	7.8	14.0	0.6	1.8	
F5	FHESFYDWFVFR.VPGGSSGGSQL.CVQE <u>E</u> EL <u>F</u> E <u>G</u> D <u>S</u> L <u>G</u> <u>D</u> <u>C</u> SG	16.8	7.3	12.9	0.6	1.8	
F10	FHENFYDWFDRQVSGGGGGVLDERF.GAC <u>P</u> <u>S</u> GY <u>C</u> SG	13.9	5.9	10.8	0.5	1.8	
E10	FHENFYDWFDRQVSGGGGGVLDERF.GAC <u>P</u> <u>S</u> GY <u>C</u> SG	10.6	5.1	8.9	0.6	1.8	

FIG. 44B-1

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Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F4	FHDNFYDWFVFR.VAGGSGGGGSHL <u>CV</u> PEELFWGASLFGY <u>C</u> SG	15.7	3.1	5.6	0.6	1.8
H2	FGEDFYDWFVFR.VSGCGGCGGSHL <u>CV</u> LD <u>EL</u> FWDASPF <u>G</u> CP <u>G</u>	11.4	2.5	4.6	0.6	1.8
E11	.HDNFYGYWFDRQVSGGSGGGSHL <u>CV</u> LD <u>EL</u> FWGASLFGY <u>C</u> SG	11.7	1.3	2.2	0.6	1.7
F12	FQENFYDWFVFR.VSGDELGGAS <u>Q</u> GG <u>C</u> SG	10.6	7.0	9.6	0.7	1.4
F9	SHESFYDWFVFRQVSGGSGGGSD <u>L</u> <u>C</u> W <u>E</u> EL <u>G</u> AP <u>V</u> G.GSS	16.0	9.9	13.3	0.7	1.3
E4	FOPENFYDWFDRQVSGGSGGG <u>C</u> SS <u>G</u>	16.4	13.4	15.8	0.8	1.2
H10	FRENFYDWFERQVSGGSGGGSHL <u>CV</u> LE <u>E</u> LSWGAS <u>T</u> FG <u>S</u> CS <u>G</u>	10.8	7.8	9.1	0.9	1.2
F3	IHVDFYDWFAR.VSGGGGGGG <u>S</u> SHL <u>CV</u> LD <u>EL</u> FWDASLF <u>G</u> D <u>C</u> AG	14.2	3.9	4.6	0.8	1.2
G6	FHASFYDWFDRQVSGGSGGGSHL <u>CV</u> LD <u>E</u> GL <u>F</u> WGAA <u>P</u> FGY <u>C</u> SG	16.2	11.0	12.1	0.9	1.1
H3	SDANFYDWFRL.VSGGSGGGSHL <u>CA</u> LEE <u>Q</u> FWDASLF <u>G</u> D <u>C</u> SG	13.1	9.8	11.1	0.9	1.1
G5	FHDKFYDWFVFS.VAGGSGGGGGSHL <u>CV</u> LED <u>R</u> FWG <u>S</u> SS <u>L</u> SGY <u>C</u> SG	14.7	7.1	7.9	0.9	1.1
H9	FHDNFYDWFVFRQVT <u>D</u> SGGGGGSQL <u>C</u> VVED <u>EL</u> FWDASR <u>F</u> GY <u>C</u> .G	13.1	8.2	8.0	1.0	1.0
G3	VSEDFYEWFR.ASGGSGGGGSN <u>L</u> <u>C</u> VE <u>E</u> EL <u>F</u> WG <u>S</u> SL <u>I</u> G <u>D</u> <u>C</u> SG	13.7	11.7	2.5	4.6	0.2
G4	FOPENFYDWFVFRQVSGGSGGGSHL <u>CV</u> LE <u>E</u> EL.u.WGASMF <u>G</u> Y <u>C</u> SG	10.0	4.3	0.7	6.0	0.2
F6	FQENFYDWFVFRQVSGGSGGGSHL <u>CV</u> LE <u>E</u> AL <u>F</u> WG <u>S</u> SL <u>F</u> LG <u>G</u> . <u>C</u> SG	5.6	9.0	0.4	21.2	0.0

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Non-Binders:

H1	DYKDGRGGRRF.GRSSVVLMKRL.R	1.2	0.7	0.5	1.5	0.7
G10	DTKTFIGITGVLPRLSAV.GFWGGSW	1.7	0.3	0.3	0.8	1.2
G9	CHENFYWWFVSQVAGGSGGGSGSRLCIM.ELF <u>F</u> RGASLFGY <u>S</u> SG	2.0	0.4	0.5	0.9	1.1
F1	FHANFYDWFVFR.VSGGSGGGGGSHL <u>CV</u> LE <u>E</u> LVSGP <u>S</u> LL <u>G</u> Y <u>C</u> SG	14.5	0.6	1.5	0.4	2.3
H8	FHEKFYDWFDL.LSGGGSGGGSHL <u>CV</u> RE <u>E</u> PF <u>W</u> GA <u>S</u> LF <u>G</u> Y <u>C</u> PG	9.7	0.6	1.5	0.4	2.3

FIG. 44B-2

IGFR Binders with change in Cys

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F8 (X14)	HLCVLEELFWGASLFGYCSG					
D8 (X6)	WLDQEWAIVQCEVYGRGCP					
G1 (X4)	SHDNFYDWFWFVR. VSGGSGCCGSP1 <u>C</u> VIGNCSEG	11.3	2.8	10.6	0.3	3.8
E8 (X6)	FYDNFYFYDWFWFDR. VSGGSGCCGSSH1 <u>C</u> VLEERV <u>C</u> GAS1 <u>C</u> FDYRSG	13.5	0.9	3.4	0.3	3.7
E3 (X10)	FHENFYDYDWFWDRQVSGGSSCGSSH <u>C</u> QEERFWGAS1 <u>C</u> GY <u>C</u> SG	15.9	1.9	6.7	0.3	3.5
F8 (X6)	FHGDFYDWFWFVR. VSGGSGCCGSSH1 <u>C</u> VLEELY <u>C</u> SG	13.7	3.6	9.5	0.4	2.6
E1 (X4)	FQDNFYDYDWFWFVRQVSGGSGCCGSSH <u>C</u> RY <u>C</u> LEG <u>C</u> SG	13.4	5.8	13.3	0.4	2.3
H12 (X4)	FHENFYDYDWFWDRQVSGGSSA <u>C</u> LFGY <u>C</u> SG	9.8	3.9	8.5	0.5	2.2
H6 (X3)	VHESFYDWFWFVR. VAGGSGCCGSSH1 <u>C</u> DVD <u>C</u> SG	11.5	4.8	9.6	0.5	2.0
E10 (X4)	FHENFYEWFWDRQVSGGSGGVLDERF. GAC <u>C</u> PSGY <u>C</u> SG	10.6	5.1	8.9	0.6	1.8
F12 (X2)	FQENFYDWFWFVR. VSGDELSSGGASQ <u>C</u> GSC <u>C</u> SG	10.6	7.0	9.6	0.7	1.4
F9 (X5)	SHESFYDWFWFVRQVSGGSGCCGSSD1 <u>C</u> WEELCGGAPLVG.GSS	16.0	9.9	13.3	0.7	1.3

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IGFR Binders with loss of F8

G7	FHANFYDWFWFVRQV. GGSCGGGGSG	16.1	2.1	9.8	0.2	4.7
F2	YHENFYDWFWFVR. VSGGSG	14.4	6.2	12.8	0.5	2.1
E7	FHENFYDWFWFVRQVSGGSGGGSG	15.4	7.8	14.0	0.6	1.8
E4	FHENFYDWFWDRQVSGGSGGGSGSS	16.4	13.4	15.8	0.8	1.2

IGFR Binders with loss of Cys in F8

F11	FHENFYDWFWFVRQVSGGSGGGSGSHL.GSG	12.5	0.8	5.2	0.2	6.5
H7	.HENFYDWFWFVRQVSGGSGGGSGSHLFGYGS	7.2	0.6	2.7	0.2	4.5
F7	FHENFYDWFWFDRQVGGGGGGSPVRTGRTVLLGFSVRLLLW	15.0	2.7	10.9	0.2	4.1
G2	FHENFYDWFWFDRQVSGGSGGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5
H4	FHDNFYDWFWFDRQVSGGSGGGSGSPFG.RSD	11.2	5.3	10.0	0.5	1.9

FIG. 44B-3

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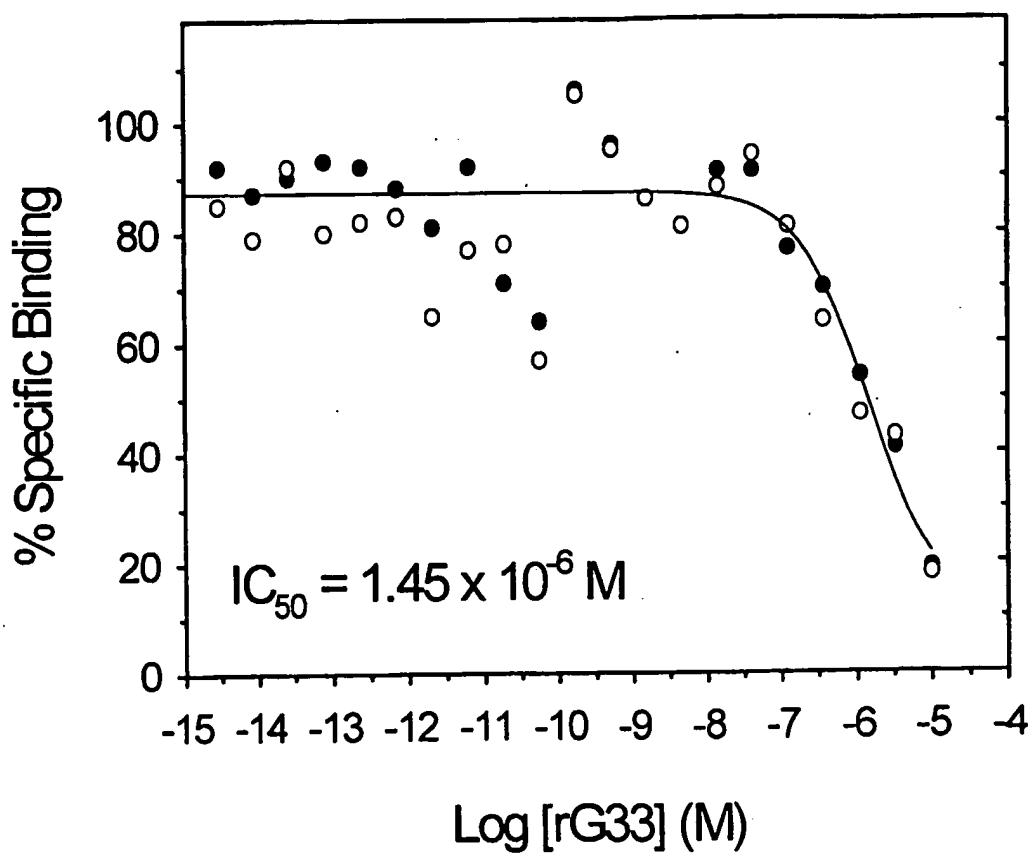


FIG. 45

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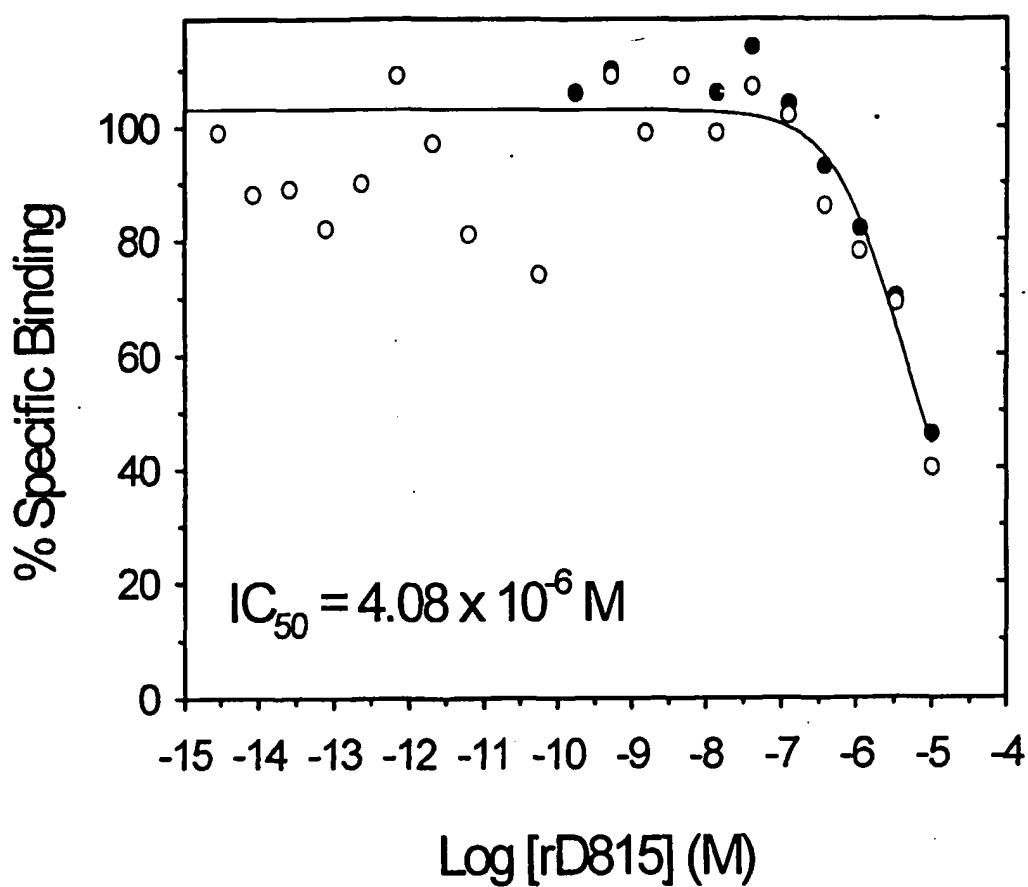


FIG. 46

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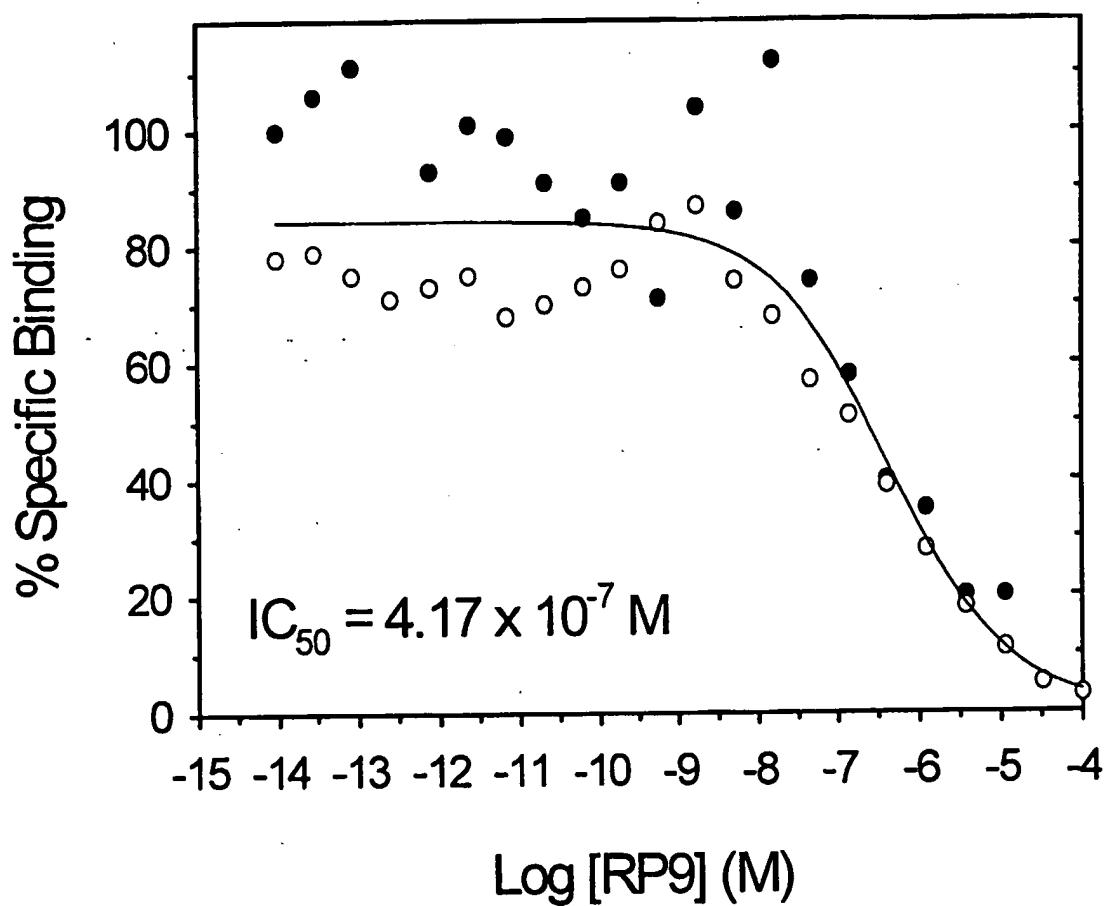


FIG. 47

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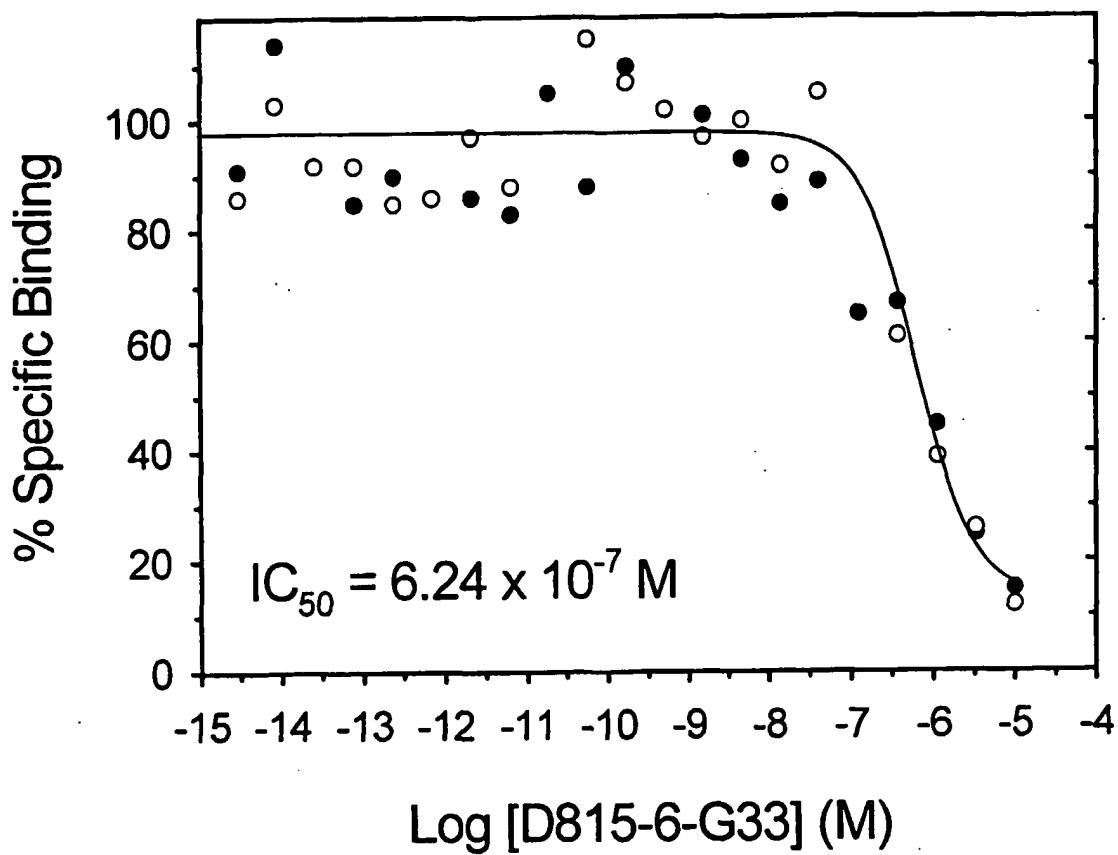


FIG. 48

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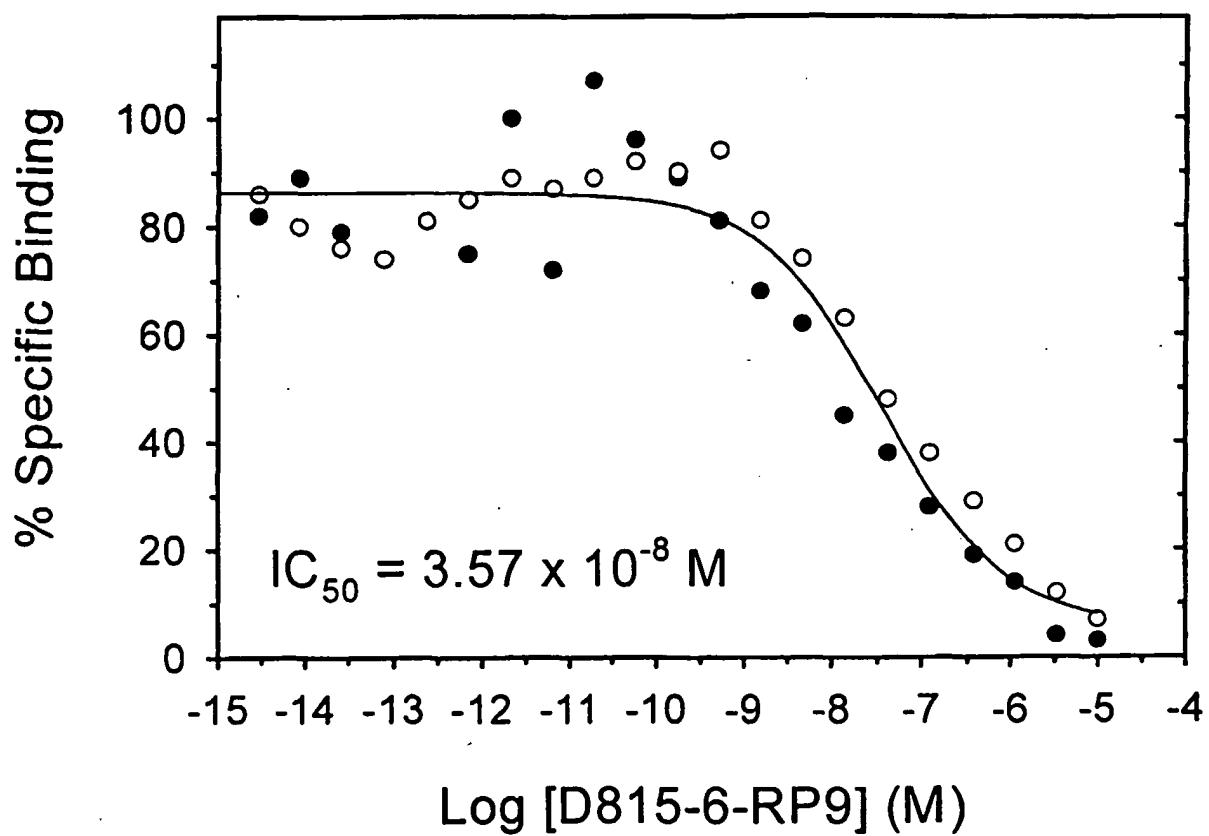


FIG. 49

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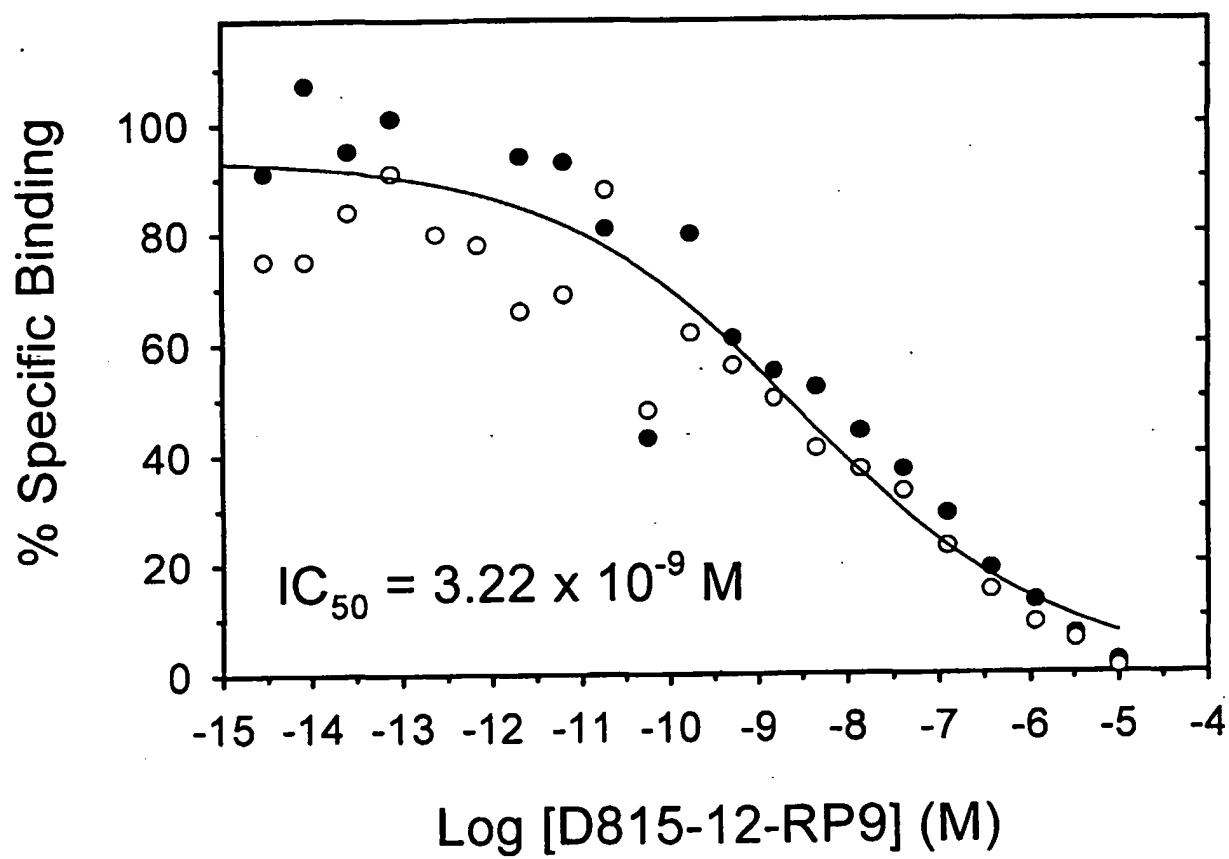


FIG. 50

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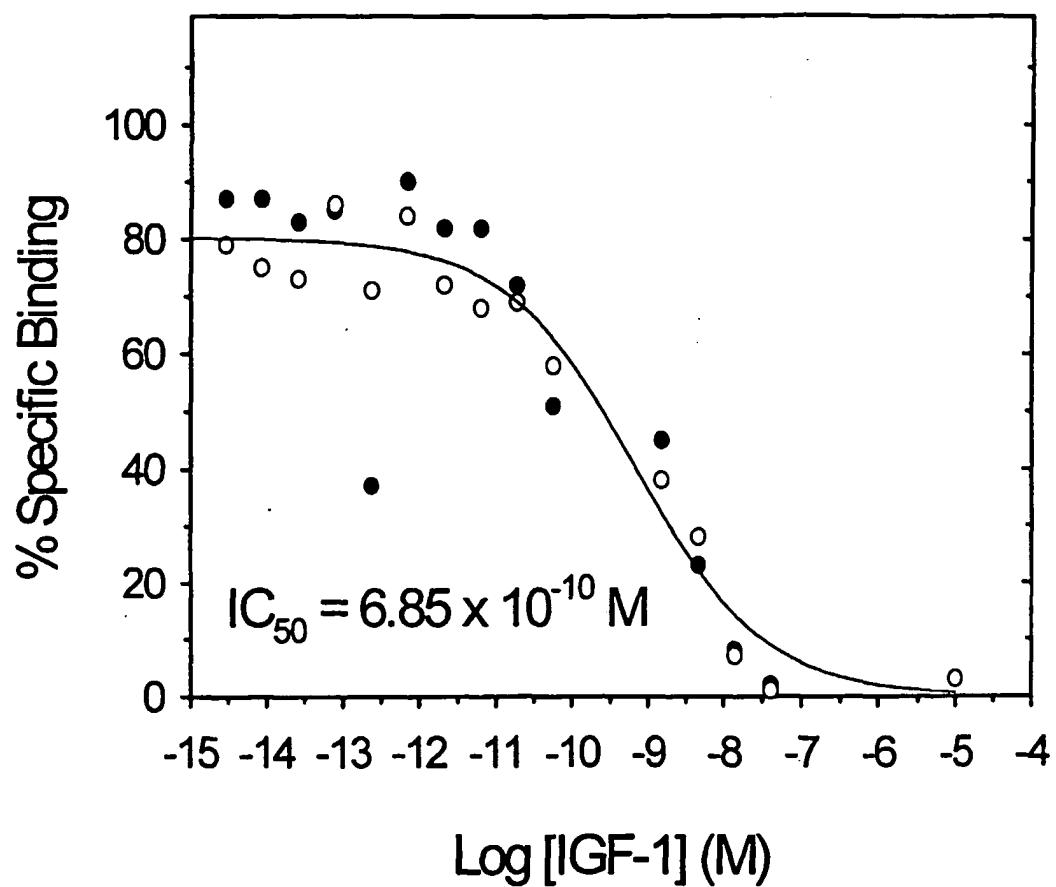


FIG. 51

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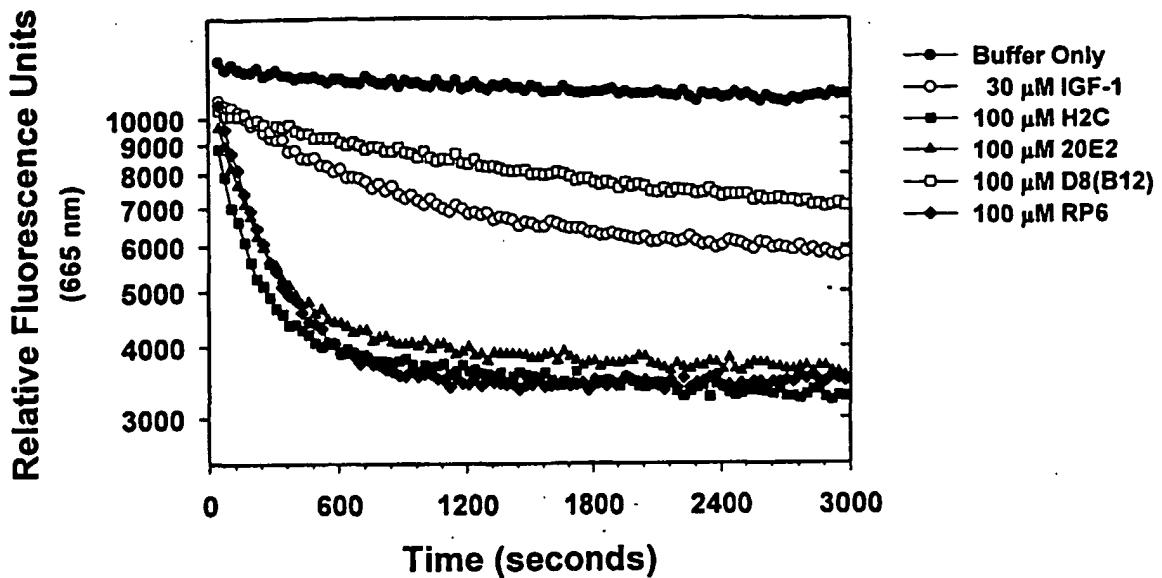


FIG. 52

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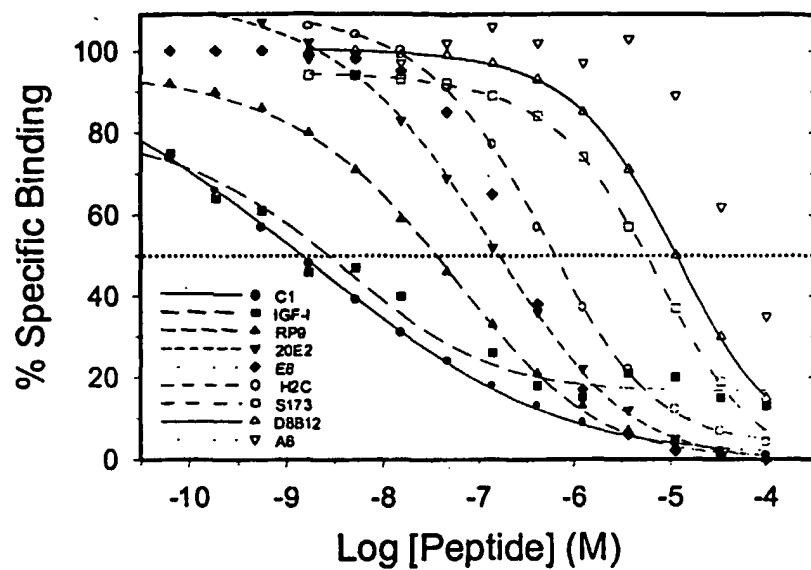
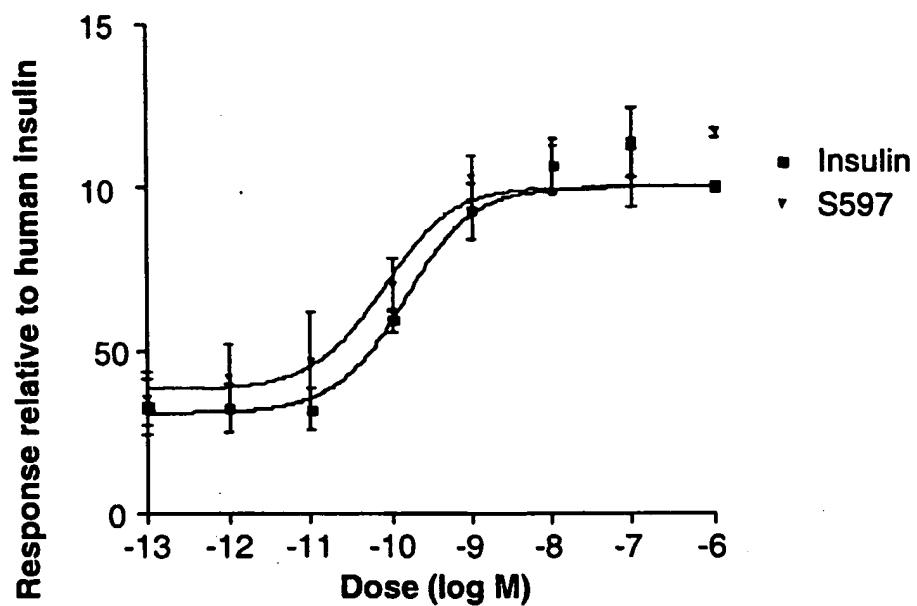


FIG. 53

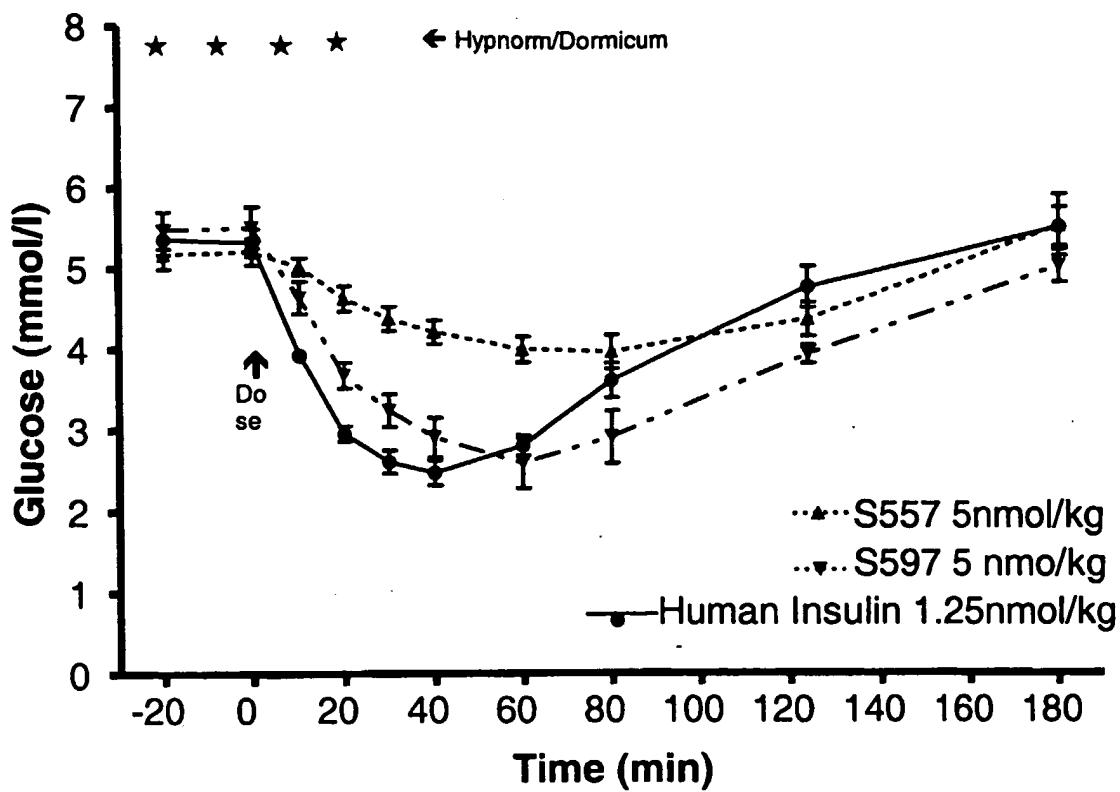
Figure 54:

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Figure 55:



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Figure 56:

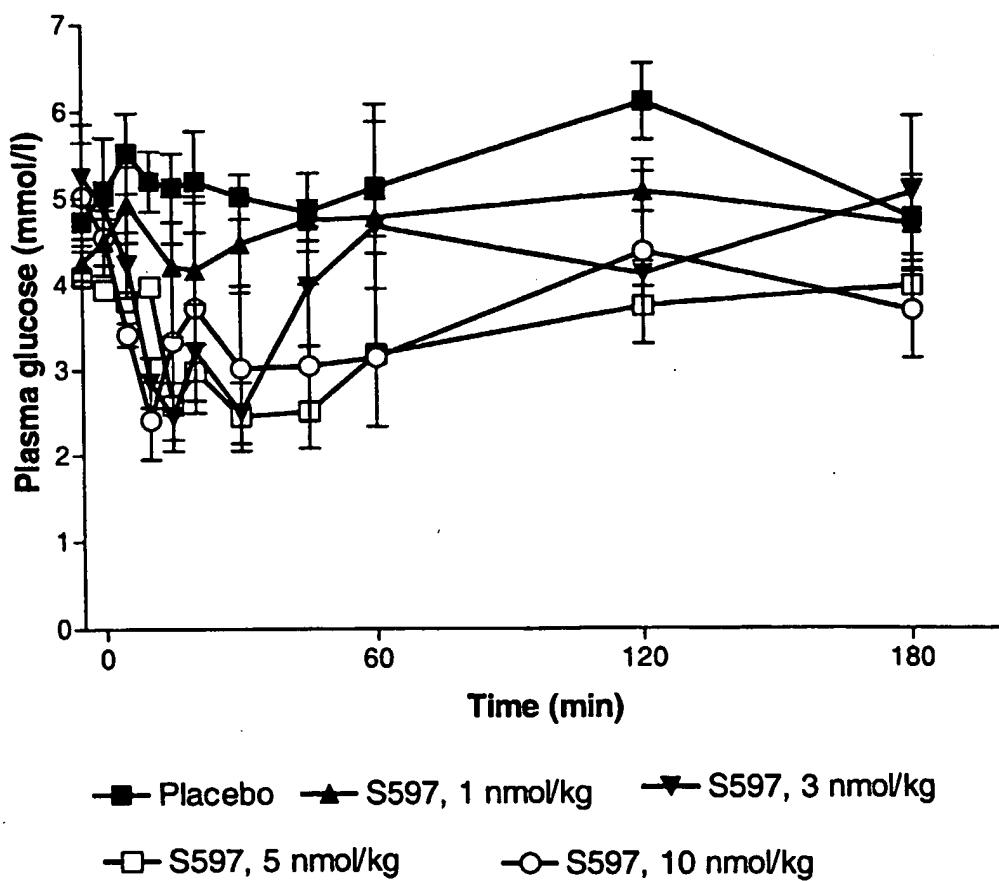


Figure 57

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